Application of the Spinning Mesh Disc Reactor for Process Intensification

Shivaprasad, Parimala

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Submitted by

Parimala Shivaprasad

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Abstract

Conventional chemical processing methods are challenged by inefficient process scale-up resulting in high energy consumption and waste generation. Process intensification (PI) is a promising solution to enable a quantifiable change in reaction engineering through development of novel reactors and/or processing methods. The spinning mesh disc reactor (SMDR) is an innovative catalytic reactor and has so far shown to intensify enzymatic reactions in an aqueous medium. The aim of this research is to further extend the application of the SMDR, by evaluating a range of reaction systems of commercial importance and currently challenging to scale-up using traditional reactors.

Three main reaction systems have been reported in the present study: (i) nitroaldol (Henry) reaction (metal catalyst-organic solvent), (ii) kinetic resolution of a racemic alcohol (lipase catalyst-organic solvent) and (iii) solar light driven photo-oxidation (photocatalyst-aqueous system). The SMDR was first investigated for Henry reaction using copper triflate immobilised on wool. A maximum conversion of 93% was achieved at the end of 5 hours in the SMDR with an improved reaction rate compared to the batch system. The application of the reactor was then extended for kinetic resolution of racemic 1-phenyethanol using an inexpensive amano lipase immobilised on wool as a catalyst. The productivity in the SMDR using the lipase catalyst cloth increased by 30% compared to the reaction in batch and the throughput was also successfully scaled up in the reactor without a loss in the reaction efficiency. Two different routes to SMDR scale-up was investigated using the above optimised reaction systems and results showed an improved reaction rate with either an increase in cloth size or cloth number. A further improvement in rate was observed with multiple cloths of increasing cloth diameters at higher substrate concentration. The last part of the research work presented was carried out at TU Eindhoven, where a modification of the SDR, the rotor-stator spinning disc reactor (rs-SDR) was used to investigate solar light photocatalytic reaction. The reactor was used to demonstrate the proof of concept for a multi-phase, photocatalytic oxidation of L-methionine using methylene blue catalyst. The reactor performance was characterised based on its flow regimes and the productivity in the reactor was found to be four times higher compared to the batch reactor.

Overall, to the best of the author's knowledge, this is the first study which has reported the use of a novel catalytic reactor for intensification of organic, biochemical and photocatalytic transformations of commercial importance, hence demonstrating the reactor's potential for enabling process scale-up at a pilot scale.

Acknowledgement

Obeisance to the Almighty for His blessings.

They say a PhD is a journey where you end up with a lot more than just the title to your name. I couldn't have agreed more! The last 3.5 years has been an eventful journey with lots of new experiences which has transformed me to a great extent as a researcher and more importantly as a human being. Although a PhD is an independent degree, mine wouldn't have been as enriching an experience without the amazing people who were there with me throughout the last few years. Getting a PhD in the UK was my dream, but it wouldn't have been possible to realise it on my own!

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During my PhD, I also discovered an entrepreneurial side to me. I had an idea for sustainable floral waste management in India, but little did I know I would be working on pursuing it as a social enterprise. This would've been impossible without the amazing team at SETSqaured Bath. I am

forever thankful to Siobain and Rosie, who showed faith in my business plan and encouraged me to seriously consider developing the idea into an enerprise. A big thanks to all the mentors who have guided me so far and the rest of the team at the centre who do a fantastic work supporting graduate entrepreneurs. Thanks also to the Alumni who sponsored my Innovation Award for the year ahead and funding my enterprise at various stages. I am now looking forward and excited about this part of my life.

Bath has been a wonderful city to live and work in, but it also gave me friends whom I will treasure for life. To Pranit, thank you for braving my cooking experiments, MBA crash course, late night philosophy and those reflective walks around the city. I don't think I would've thought about being an entrepreneur without watching you do it all! To my first friend in Bath, Sriharsha, thank you for all the good advice and the help with absolutely everything from the beginning, Trinity Road and political debates on the way back home! Life in Bath wouldn't be the same without you both. A big thank you to Deepak, Amit, Saravanan (times 2!), Sameer, Abouther, Dan (my constants when I had to work over the weekends!), Ida, Sandhya, Hitesh, Dibya, Jahnavi, Fei, Zoe, Adrien, Joy, Nathan, Chris and Donna who have made living in Bath a memorable experience. Special shout out to Lola, Poppy and Rossie!

I spent the last 6 months of my PhD at TU Eindhoven and it was a wonderful learning experience. I would like to thank Dr. John van der Schaaf and Dr. Tim Noel for hosting me in their group. Thanks must also go to my co-workers on the project Koen and Arnab. I also thank the entire SPE-CRE group for welcoming me into the group which made my stay in Eindhoven a very memorable one.

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Nomenclature

PI	Process Intensification
SDR	Spinning Disc Reactor
SMDR	Spinning Mesh Disc Reactor
PI	Process Intensified Enzymatic Reactors
DKR	Dynamic Kinetic Resolution
rs-SDR	Rotor-Stator Spinning Disc Reactor
BSTR	Batch Stirred Tank Reactor
RTD	Residence Time Distribution
PEI	Polyethylene imine
FTIR	Fourier Transform Infrared
UV-Vis	Ultraviolet-Visible light
XPS	X-Ray Photoelectron Spectroscopy
SEM	Scanning Elctron Microscopy
EDX	Electron Dispersive X-Ray
NMR	Nuclear Magnetic Resonance
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
ee	Enantiomeric Excess
R and S	Fractions of enantiomer in a mixture
S	Shear stress (s^{-1})
Q	Volumetric flowrate ($m^3 s^{-1}$)
R	Radial distance (m)
ω	Angular velocity (rad s^{-1})

v	Kinematic viscosity (m^2 s^{-1})
RPM	Rotation per minute
f	Film thickness
ρ	Liquid density
t	Residence time

Chapter 1

Introduction

Conventional reactors like the stirred tank reactors have been used for chemical processing for close to 500 years with little or no change to the reactor design. While they are easy to operate and an economic solution for operation on a small scale, they are met with significant challenges related to multiphase reactions and reactor scale-up [1]. Other reactor configurations like the packed bed reactor and bubble column reactors have shown better performance for multiphase reactions with improved catalyst recovery, but are still limited in terms of high heat and mass transfer resistance upon scale-up. Chemical industries today are receptive to finding sustainable alternatives for chemical processing over conventional methods. For any process to be sustainable it is imperative to identify and choose the right chemical transformation, suitable raw materials and catalysts, design a suitable reactor prototype, scale-up and optimise the process for commercial scale production [2]. Hence, there is a need for multi-functional reactors and alternate processing methods which can alleviate intermediate, inefficient processing steps and reduce the need for energy intensive downstream product purification.

1.1 Process intensification

Process intensification (PI) is a recent development in chemical processing with a potential to facilitate a quantifiable change in the conventional manufacturing practices of chemical industries. This is usually accompanied by a reduction in the size of the apparatus, energy consumption and/or waste generation, resulting in a sustainable development of process industries [3]. On a macroscale, PI has led to a new generation of reactors and processing methods (Fig 1.1), allowing for better control of the reaction pathways at meso and molecular level leading to: (i) enhanced reaction rate (ii) increased selectivity of the product and (iii) scale-up of novel chemistry on a commercial scale [5]. An intensified process or a reactor necessarily incorporates one or more fundamental domains of PI [4, 5], namely, spatial, thermodynamic, functional and temporal domain (Fig 1.2). Among the new generation of PI reactors, the spinning mesh disc reactor (SMDR) has shown potential to incorporate the fundamental domains of PI and facilitate reaction intensification. The present study is focused on further extending the application of the SMDR as a PI reactor.

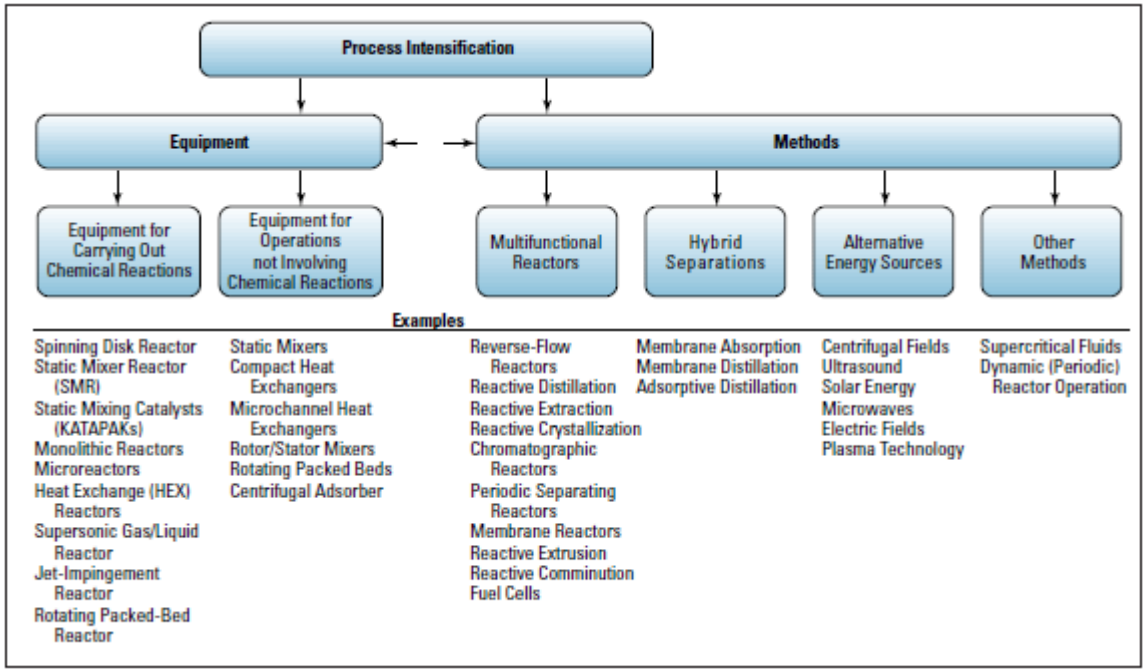

Figure 1.1: The components of process intensification [4]

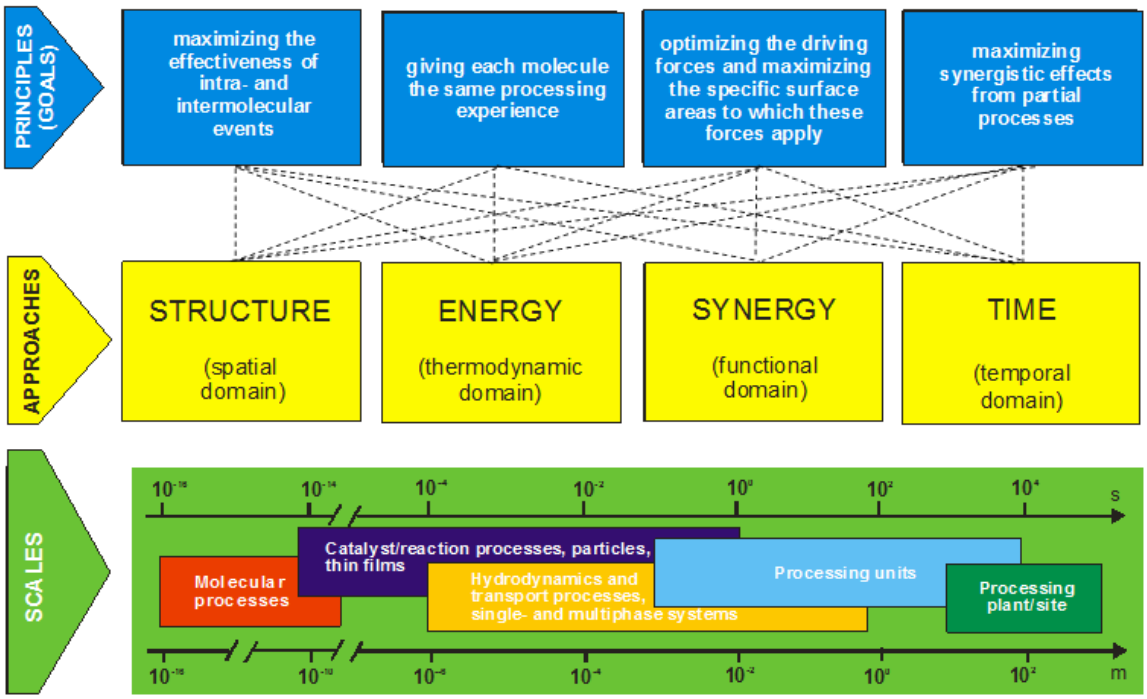

Figure 1.2: Various domains of process intensification [6]

1.2 The spinning mesh disc reactor for process intensification

The spinning mesh disc reactor (SMDR) is an innovative reactor design which uses centrifugal force (like the spinning disc reactor) for an even spread of thin film on the surface of the disc,

which additionally holds a cloth immobilised with a catalyst. The reactor can incorporate the fun-

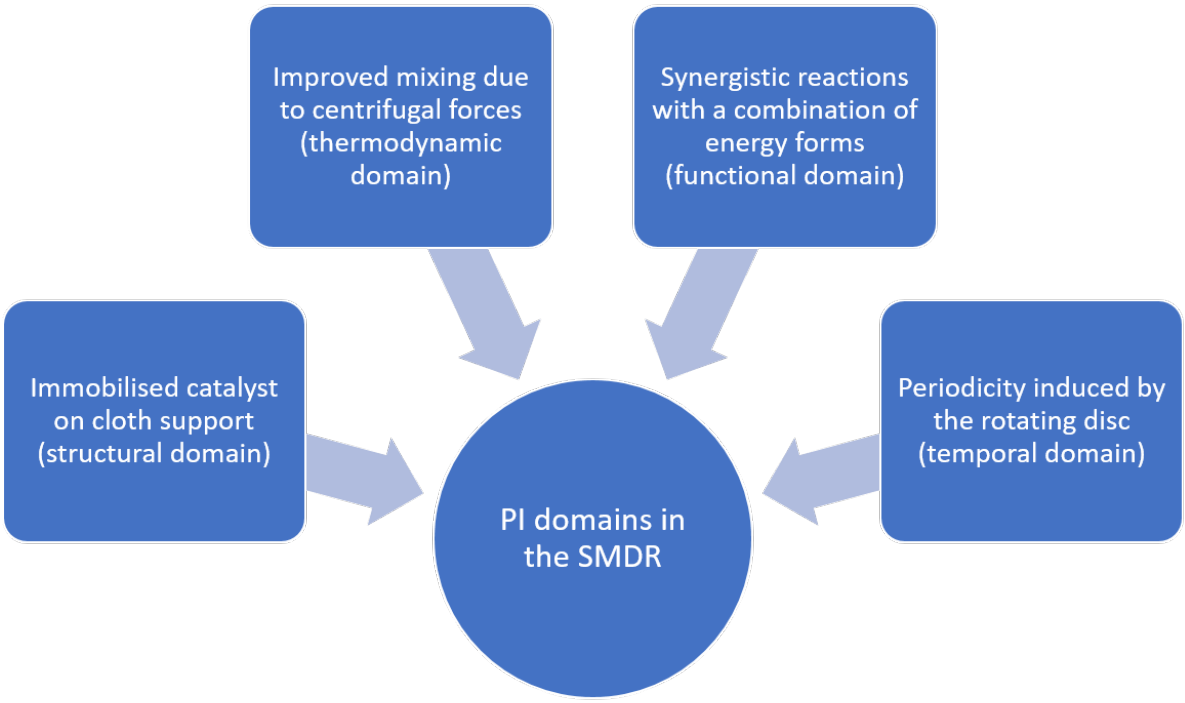

Figure 1.3: Domains of PI in the SMDR

damental domains of PI as shown in Fig 1.3. The structural element of the spatial domain can be observed in the SMDR as it can house catalyst immobilised on a cloth support like wool. The reactor can incorporate the thermodynamic domain as it has shown to promote better mixing compared to conventional reactors due to the centrifugal force associated with the disc. The locally generated high gravitational fields also selectively activates the reactant molecule improving the performance of the reactor. Functional domain in the reactor can be enabled through synergistic reactions on a meso scale, e.g. a photocatalytic reaction, which is a combination of gravitational energy from the spinning disc and light energy. This also helps with selective activation of the catalyst without the unnecessary temperature rise in the bulk of the reaction. The periodicity in the SMDR is induced by the spinning disc, which in turn increases the interfacial mass transfer, thus fulfilling requirements of a temporal domain. Further, for reactions coupled to a light source, dynamics can also be introduced in terms of varying the intensity of light, which influences the orientation of the molecules and the reaction rate kinetics.

Feng et.al [7] have reported the use of the SMDR (Fig 1.4) for hydrolysis of tributyrin using lipase immobilised on wool and benchmarked the enzyme activity and conversion against a conventional Batch Stirred Tank Reactor (BSTR). One of the major drawback of BSTR is enzyme

de-activation as free enzyme denatures in solution and the activity reduces on re-use. This was

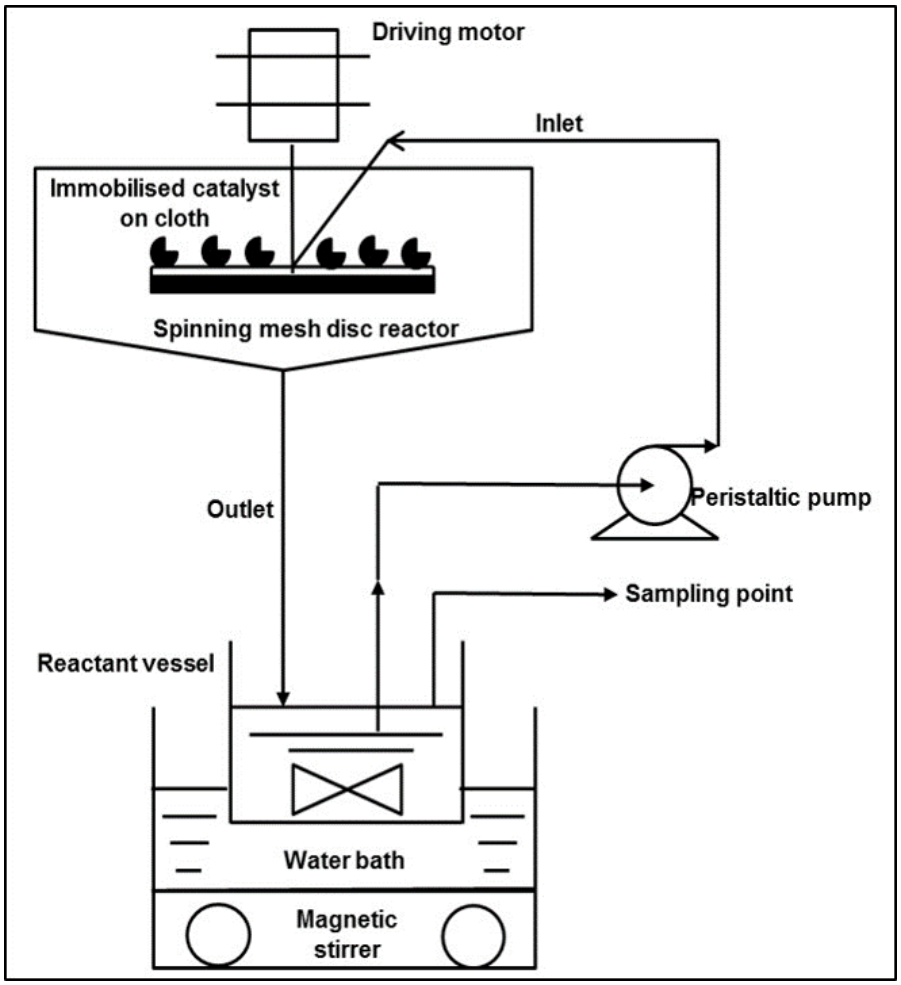

Figure 1.4: Schematic diagram of the spinning mesh disc reactor (SMDR) used in the present study

successfully overcome by immobilizing the enzyme on wool, which provided better catalyst stability, re-usability and enhanced product separation. In addition to protecting the enzyme from hydrodynamic forces, the cloth also increases the rate of mixing and hence an increase in the reaction rate as a highly sheared thin liquid film flows on top and within the cloth. Maximum conversion of 72% was observed for a flow rate of 5 ml s^{-1} and a disc speed of 400 RPM due to rapid mixing within and on top of the cloth. With an increase in the disc speed to 500 RPM, the conversion reduced to 69%. This could be due to an increase in shear force which either deactivates the enzyme or causes enzyme leaching. The residence time decreases with increase in the disc speed which results in a reduced contact between the enzyme and the reaction mixture. The activity of immobilised enzyme was studied by reusing the lipase immobilised cloth for 15 cycles and the authors reported a good enzyme activity of 80% after the entire run [8]. In addition to being an intensified technology, SMDR also holds the promise of stable immobilised catalytic reactions on the industrial scale.

Examination of RTD studies illustrated that under most cases of spinning speed and flowrate, the reactor deviated from plug flow (N<50) in difference to a conventional SDR. The improved mixing in the SMDR can be attributed to the equivalent number of tanks in series, which is two times lower than the reactor without the cloth. At low spin speed and high flow rate, visual observations (Fig 1.5) indicated radial flow on the disc which was uneven with zones of dye-free region. The radial flow was replaced with concentric flow on the disc at high spin speed and low flowrate. A

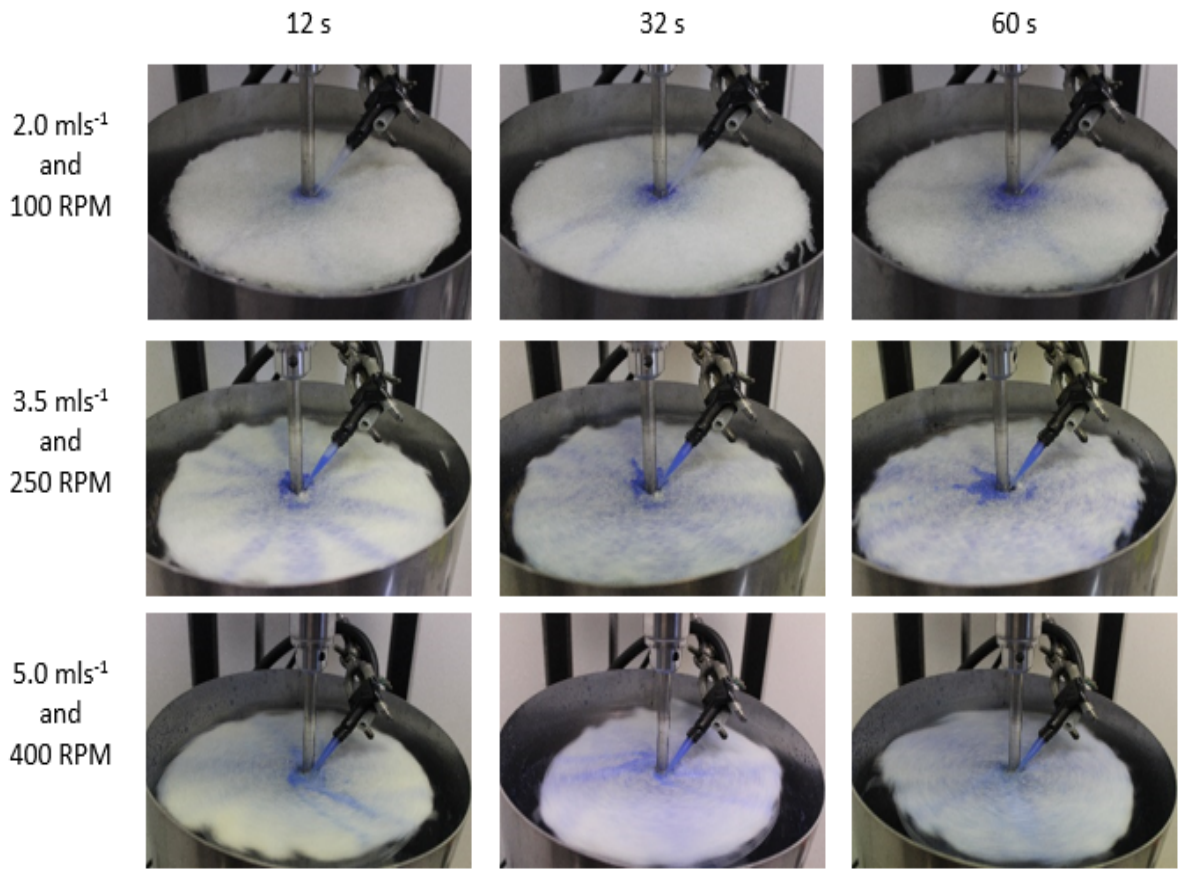

Figure 1.5: Flow regimes on the disc surface at different spinning speed and flowrate

well-mixed model was used to simulate the hydrolysis of tributyrin in the SMDR. The volume of liquid on the cloth is surface is small compared to the volume in the reaction vessel, which well mixed. Hence, the overall mass balance can be expressed as:

$$\frac{dC}{dt} = -\frac{V_r}{V_t} r' \tag{1.1}$$

Where, V_r is the liquid volume in the reactor (m^3), V_t is the total liquid volume in the given system (m^3), r' (mol m^{-3} s^{-1}) is the overall reaction rate and C is the initial substrate concentration (mol m^{-3}).

The volume of the liquid in the reactor can be expressed as an integral of the film thickness as it is

assumed that fully developed laminar films are developed on the surface of the cloth [9].

$$V_r = \left(\frac{81\pi^2 Q\nu}{16\omega^2}\right)^{1/3} R^{4/3} \tag{1.2}$$

Where, R = radius of the disc (m); ν = kinematic viscosity ($m^2\ s^{-1}$; Q = volumetric flow rate ($m^3\ s^{-1}$); ω = angular velocity (rad s^{-1})

The global mass transfer co-efficient for an immobilised catalyst can be expressed as:

$$k_L a(C - C_i) = r' \tag{1.3}$$

where, k_a is the global mass transfer co-efficient (s^{-1}), C is the substrate concentration in the bulk of the solution and C_i is the initial substrate concentration (mol m^{-3})

Enzymatic hydrolysis of tributyrin has been shown to follow Ping Pong Bi Bi mechanism as follows:

$$r' = \frac{\frac{v_{max}}{K_m} C_i}{1 + \frac{(C_0 - C_i)}{K_i}} \tag{1.4}$$

where, r' is the reaction rate, v_{max} is the maximum rate of the reaction, K_m and K_i are the kinetic constants, C_0 is the final concentration of the substrate and C_i is the initial substrate concentration.

Substituting equations (1.2) to (1.4) in equation (1.1) and solving for $k_L a$, it was found that 0.6 to 1.3 s^{-1}, and 0.769 to 1.462 s^{-1} as the spinning speed increased from 250 to 500 rpm for the flow rates of 2 mL s^{-1} and 5 mL s^{-1} respectively [10]. Further, the above model was used to fit the time course of tributyrin hydrolysis in the SMDR and a good fit was obtained between experment and calculated values indicating that the SMDR behaves like a well-mixed reactor [10]. Although this is undesirable in an SDR, the mixing produce in the SMDR is beneficial for increasing the contact between the feed and the catalyst immobilised on the cloth support.

Scale-up of the SMDR was easily achieved by increasing the number of lipase immobilised cloths on the disc. An increase in conversion was evident as the catalyst loading per unit volume of the reaction mixture increased [11]. This further proves that scale-up of a SDR in general does not require a recourse of operating co-efficient like Prandtl and Reynolds numbers but is solely governed by physical laws [12].

1.3 Objectives

The SMDR has been used till date only for enzymatic reactions in an aqueous system and has shown proof of concept as a PI reactor. The aim of this thesis is to further extend the application of the reactor for a range of novel chemistries of commercial importance which would benefit from improved reaction engineering. Three main reaction systems were chosen in the present study:

(a) Organic synthesis with a metal catalyst

(b) Biochemical synthesis with a lipase catalyst

(c) Solar light driven photocatalytic reaction

Wool was used as a catalyst support material for all reactions in the SMDR after screening a range of support materials (see chapter 2), as it was found suitable for the high shear reactor environment. The specific objectives of the present study are:

1. Nitroaldol condensation reaction (Henry reaction) will be used to investigate the application of the SMDR for organic reactions. This is a challenging reaction as it is limited by mass transfer limitations in batch and has only been carried out at a scale of $\sim$100 ml till date and hence can justify the use of an intensified reactor like the SMDR for reaction scale-up. Also, resolution of the end product from the reaction forms an important precursor for the pharmaceutical industry. This reaction scheme can be enabled in one-pot using two different catalysts for the Henry reaction (chemo catalyst) and the resolution step (enzyme catalyst). Hence, the Henry reaction was chosen to also enable a one-pot reaction in the SMDR. This is yet another challenging reaction as maintaining catalyst isolation is key to achieve a cascade reaction. The SMDR has the potential to augment this chemistry as the catalysts are immobilised on different supports on the disc.

2. In order to mimic the resolution step of the Henry reaction and also test the reactor performance for enzyme catalysed organic synthesis for the first time, lipase catalysed kinetic resolution of 1-phenyl ethanol will be investigated next in the SMDR. This has so far been carried out using expensive enzymes with poor recoverability and at a scale of $\sim$50 ml and hence can further demonstrate the potential of the SMDR for intensification of enzyme catalysed organic reactions. The one-pot kinetic resolution reaction will then be tested in batch and SMDR

3. The scale-up in SMDR has been previously demonstrated by addition of catalyst cloths to increase the catalyst loading for enzymatic tributyrin hydrolysis. Catalyst loading can also be increased by increasing the cloth size by using different disc diameters and this has not been investigated so far. Hence, a newly designed SMDR has been used to evaluate the reactor performance by increasing cloth size and/or cloth number. Enzymatic hydrolysis of tributyrin and Henry reaction have been used for this study. The application of copper triflate catalyst will also be investigated for oxidative homocupling of benzylamine in the SMDR, further extending the reactor's potential for organic synthesis.

4. The last part of the work presented in this thesis was carried out at TU Eindhoven, where another modification of the SDR, the rotor-stator spinning disc reactor (rs-SDR) has been used

to investigate photocatalytic reactions. The photo rs-SDR (rs-SDR illuminated with a solar simulator) has been investigated for the first time to demonstrate the proof of concept for the photocatalytic oxidation of L-methionine using methylene blue. This is a challenging reaction as it is limited by light intensity and gas-liquid mass transfer resistance and hence shows the potential of a photo rs-SDR for improving the reaction efficiency. This is also an example of a synergistic reaction where two different energy forms (gravitation and light energy) provide a pathway for sustainable chemical processing.

1.4 Scope of the thesis

The research work presented in this thesis is in the form of peer-reviewed journal articles and a book chapter, which are either published or under review.

Chapter 2 outlines the application of the SMDR for the first reaction system using nitroaldol condensation as a model reaction [13]. Copper immobilised on wool has been used as a catalyst for the reaction in batch and the SMDR. Based on the review of recent advances in process intensified reactors for enzymatic reactions presented in Chapter 3 [14], the application of the SMDR was extended for enzyme catalysed organic synthesis. Chapter 4 presents the investigation of lipase catalysed kinetic resolution of a racemic alcohol in the reactor [15].The chapter ends with an attempt at carrying out a one-pot chemo-enzymatic cascade reaction in the SMDR. However, this reaction was met with some challenges and hence only a few preliminary results have been discussed. Chapter 5 provides a detailed study of the two routes to SMDR scale-up for the above optimised reaction systems. The chapter ends with the extension of the reactor for scale-up of imine synthesis, another example of a chemo catalysed organic reaction. Chapter 6 outlines the investigation of a solar light photocatalytic reaction in a rotor-stator spinning disc reactor (rs-SDR), another form of a spinning disc reactor. This work was carried out in collaboration with TU Eindhoven. The reactor has been used for the first time for a multi-phase photo-oxidation reaction. This is a proof of concept study to demonstrate synergistic reactions in a PI reactor. Chapter 7 outlines the key results from this thesis and potential areas for future work.

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Chapter 2

Nitroaldol condensation in the Spinning Mesh Disc Reactor

The application of the SMDR was first tested for organic synthesis namely nitroaldol condensation (Henry reaction) catalysed by copper triflate immobilised on wool. To develop this catalyst system, a number of key steps needed to be evaluated like immobilisation of catalyst on a suitable support, perfecting the reaction in batch, before optimising the reaction in the SMDR. In the process of identifying the right support for catalyst immobilisation, three different supports, namely, wool, carbon cloth and glass fibre were investigated.

(1) *Immobilisation on carbon cloth*

Activated carbon cloth samples were obtained from Mast carbon. The cloth was dried for 24 hours at 50°C after which it was refluxed with 6 M nitric acid for 24 hours to introduce oxygen functional groups. PEI surface modification and copper triflate immobilisation was carried out analogous by the protocol detailed for wool later in the chapter. The oxidation of the cloth was not entirely successful and post treatment, the cloth also disintegrated owing to the harsh conditions of oxidation. Plasma pre-treatment was not successful either for introducing the functional groups. Hence, carbon cloth was not investigated further as a catalyst support.

(2) *Immobilisation on glass fibre*

Glass fibre mesh (obtained from Fothergill) was treated with 4 N HCl for two hours to leach out the non-silica components. Post acid treatment, the fiberglass cloth was washed with deionised water and the copper immobilisation protocol similar to that for wool (elaborated later in the chapter) was followed. FTIR analysis showed successful immobilisation of copper on the glass fibre support. However, the glass fibre mesh also disintegrated during the process of acid pre-treatment and the catalyst could not be tested for the Henry reaction. Hence, glass fibre also was not found to be suitable for catalyst immobilisation.

(3) *Immobilisation on woollen cloth*

Woollen cloth has been utilised as a support for enzyme immobilisation and the resulting catalyst system was successfully applied for reactions in the SMDR [1]. Hence, woollen cloth support was examined for copper triflate immobilisation and initial immobilisation on

small batches of wool was found to be successful. The protocol was then scaled up for catalyst immobilisation on wollen cloth discs for application in the SMDR.The amount of catalyst used by based on the amount of copper loading determined by EDX as detailed in the following paper.

This paper investigates the scale up of the Henry reaction in the SMDR using immobilised copper triflate on woollen cloth support. The optimised protocol for immobilising copper triflate on wool has been reported followed by a full characterisation study of the copper wool. Results from batch studies have then been discussed for both free and immobilised catalyst along with the optimisation study of the reaction in the SMDR. Additional plots for this chapter can be found in Appendix A.

<table>
<tr><td colspan="10">This declaration concerns the article entitled:</td></tr>
<tr><td colspan="10">Process Intensification of Catalysed Henry Reaction using Copper-Wool Catalyst in a Spinning Mesh Disc Reactor</td></tr>
<tr><td colspan="10">Publication status (tick one)</td></tr>
<tr><td>draft manuscript</td><td></td><td>Submitted</td><td></td><td>In review</td><td></td><td>Accepted</td><td></td><td>Published</td><td>✓</td></tr>
<tr><td>Publication details (reference)</td><td colspan="9">Shivaprasad, P., Jones, M. D., Patterson, D. A., & Emanuelsson, E. A. C. (2017). Process intensification of catalysed Henry reaction using copper-wool catalyst in a spinning mesh disc reactor. Chemical Engineering and Processing: Process Intensification, 122, 550-559.</td></tr>
<tr><td>Candidate's contribution to the paper (detailed, and also given as a percentage).</td><td colspan="9">Formulation of ideas (50%): The reactor was developed by Emma Emanuelsson and Darrell Patterson and the reaction scheme presented in this paper was proposed by Matthew Jones as his group had reported the reaction in batch using a homogeneous catalyst. I proposed tests with alternate catalyst supports as a part of an initial study before choosing wool as a suitable support. I also contributed to further studying the catalytic property of the wool.

Design of methodology (60%): The co-authors suggested an outline for the methodology for reactions in batch and SMDR and I further built on the design of the experiments presented in this paper. I contributed to the basic characterisation techniques and the co-authors suggested further characterisation to make it a complete study.

Experimental work (90%): I performed all the experiments in batch and the SMDR. SEM and EDX analysis of the samples were carried out by the technical staff. I carried out most of the data interpretation with subsequent discussions with my co-authors.

Presentation of data in journal format (70%): I prepared the manuscript for the journal including the graphics in the journal format and incorporated feedback from co-authors and reviewers. Individual sections have contributions from all the co-authors which improved the overall quality of the paper.</td></tr>
<tr><td>Statement from Candidate</td><td colspan="9">This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.</td></tr>
<tr><td>Signed</td><td colspan="5">Parimala.S</td><td>Date</td><td colspan="3">02/01/2019</td></tr>
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Abstract

The spinning mesh disc reactor (SMDR) is a novel process intensification technology which uses centrifugal force to drive reaction fluid over a mesh supported catalyst on a rotating disc. The potential of the SMDR for organic synthesis has been demonstrated for the first time for Henry reaction using copper immobilised on woollen cloth mesh. A new protocol for copper immobilisation on wool has been developed producing a superior catalyst to the homogeneous copper triflate system: copper heterogenised on wool afforded a higher batch conversion (85%) (cf. 57% for the homogeneous case) in the same timeframe. In the SMDR, the reaction was more efficient than either homogeneous or heterogeneous batch reaction: with further optimisation the conversion increased from 77% to 93% as the spinning speed of the disc increased from 250 to 450 RPM at a flowrate of 3 ml s^{-1}. There was only a 3% reduction in conversion on re-use of copper wool over 3 cycles under similar experimental conditions indicating that this catalyst is robust. Pure wool was also found to have some catalytic activity for the Henry reaction, giving a maximum conversion of 85% at 450 RPM in the SMDR. However, it deactivated significantly with reuse and therefore cannot be considered a stable, robust catalyst. Overall, the results show that the copper immobilised wool in the SMDR can be used to improve the conversions for the Henry reaction and that there is therefore promise for the SMDR to be extended to other traditional solvent based reactions.

2.1 Introduction

Process intensification can be synonymous with enhanced overall reaction efficiencies, lower chemical inventory, reduced operating costs and reduction in process waste generation [2]. The spinning disc reactor (SDR) is a process intensification technology where liquid is impinged at the centre of a rotating disc, producing a thin film with a high shear stress on the surface of the disc due to the centrifugal force associated with the rotating disc [3]. This allows for short residence times, an increase in mass/heat transfer and high shear forces resulting in better mixing [4]. The SDR has been applied for fast and highly viscous reactions such as in the manufacturing of pharmaceuticals [5], production of polymers [6], precipitation processes [7], synthesis of nanoparticles [8] and biodiesel production [9].

The spinning mesh disc reactor (SMDR), an innovative reactor design, builds on the SDR concept but it also has a cloth with an immobilised catalyst resting on top of the disc surface. The cloth both protects the catalyst from hydrodynamic forces, and allows for an increase in mass transfer both on top and within the cloth, creating a zone of rapid mixing. The SMDR demonstrated an 18% increase in reaction conversion under the same experimental conditions as a conventional batch stirred tank reactor (BSTR) for the hydrolysis of tributyrin using immobilised lipase on wool as a catalyst [1]. 80% of the initial activity of the lipase cloth was retained for 15 cycles, further exemplifying the robustness of the reactor. Residence time distribution (RTD) studies show that the SMDR is a well-mixed reactor, in difference to SDR, which exhibits plug flow behaviour [10]. Scale-up of the SMDR was easily achieved by increasing the number of cloths resting on the disc, increasing the conversion with an increase in the catalyst loading [11]. Similar to the micro-reactor concept, SMDR can be scaled up by "numbering up" i.e. increasing the number of reactors or by increasing the number of discs on the rotation shaft. This is in contrast to traditional reactors where scale up requires re-design in terms of parameters such as Prandtl and Reynolds numbers, thus accelerating transfer of lab scale processes to industrial applications [3, 10, 12]. To demonstrate and investigate the versatility of the SMDR, the next step is to extend the application from aqueous enzyme catalysis to chemical synthesis in organic solvents using non-biological immobilised catalysts on cloths.

Key advantages with immobilising catalysts is the potential to facilitate catalyst recovery and re-use. This has resulted in research towards finding the most appropriate support material and immobilisation methods for a wide range of catalysts. Materials including silica, zeolites and activated carbon have been widely used as catalyst supports [13]. Cloths are an alternative, less researched, promising material for catalyst immobilisation due to their superior mechanical properties, versatility, low pressure drop and increased mass transfer [14]. Research has shown that wool is an attractive support for enzyme immobilisation as it is inexpensive, has a large surface

area (ca. 200 m^2 g^{-1}) and is rich in surface functional groups (like tyrosine, glutamic and aspartic acid groups) [1, 15–18]. However, limited literature is available on immobilisation of metals or simple complexes thereof on wool. One such report by Yuan et al [19] involves the use of palladium immobilised on wool for asymmetric hydrogenation of diacetone alcohol. The resulting metal cloth was found to be robust over several cycles and wool-Pd complex was naturally chiral resulting in the synthesis of optically pure products. Copper has also been immobilised on wool to improve its antibacterial properties [20], but the protocol involves numerous time consuming and energy intensive reaction steps.

Copper has recently been shown to catalyse the nitroaldol condensation reaction (Henry reaction) using immobilised copper triflate on silica and zeolite supports (conversions $\sim$ 70 to 90% and enantiomeric excess (ee) $\sim$ 40%) with current drawbacks such as long reaction times and reduced selectivity [21]. The Henry reaction is principally a reaction between a nitroalkane and an aldehyde in the presence of a base resulting in the formation of β-nitroalcohol, a building block for various pharmaceuticals like (S)-propranolol and Amprenavir, the HIV protease inhibitor to name a few [21]. It is an important reaction in organic chemistry as it produces a new carbon-carbon bond. Most of the conventional metal catalysts for the reaction like zinc (II), chromium (III), cobalt (II) and lanthanum (III) are either toxic, expensive or not biocompatible, which are imperative pre-requisites for drug manufacture in the pharmaceutical industries [22]. Copper is cheap, bio-compatible, and relatively harmless and thus has the potential to be the metal of choice for Henry reaction.

The aim of this paper is thus (i) to demonstrate the versatility of the SMDR, (ii) to develop a simple, effective and reusable immobilisation protocols for copper on wool and (iii) to enhance the selectivity, 'ee' and the reaction rate for the Henry reaction. The copper wool will be fully characterised, and the reaction rates, selectivity and 'ee' will be compared between batch reactions and the SMDR for both plain wool and copper wool. The effect of operating parameters in the SMDR such as spinning speed and flow rate will be investigated and optimised.

2.2 Materials and Methods

2.2.1 *Materials*

All chemicals and solvents were purchased from Sigma Aldrich and used as received unless mentioned otherwise. Wool (unbleached cloth of 1.5 mm thickness) was procured from Treliske (Otago, New Zealand). All solutions were prepared using deionised water (Reservoir-Elga).

2.2.2 *Catalyst immobilisation on wool*

A four step procedure was used to immobilise copper triflate on wool. Woollen cloth was cut into circular piece of diameter 12 cm, each weighing 12 g. The cloth was first treated with a solution containing hydrogen peroxide (30 %, 30 ml l^{-1}) and sodium silicate (2 g l^{-1}) in a pH 9 buffer (0.1 M sodium bicarbonate and sodium carbonate) for 70 min at 55°C. The cloth was washed with deionised water thrice (5 minutes for every cycle) and air dried. The bleached cloth was then soaked for 2 hours at room temperature in a 2 % (w/v) solution of polyethyleneimine (PEI) (adjusted to pH 8 using hydrochloric acid) and washed with deionised water. Post surface modification with PEI, wool was soaked in a copper triflate solution in methanol (1 mM) for 24 hours followed by immersing the cloth in a 0.5 % (w/v) glutaraldehyde solution in pH 6 buffer (0.1 M sodium hydrogen phosphate and sodium dihydrogen phosphate) for 10 minutes for crosslinking and rinsed with deionised water.

2.2.3 *Material characterisation techniques*

In order to analyse the effect of pre-treatment and the reaction on wool, Fourier transform infrared spectroscopic analysis (FTIR) was carried out using a Perkin-Elmer-100 FTIR spectrometer. The plain and copper wool samples were scanned from 4000 to 600 cm^{-1} with 16 scans per wavelength. The background spectrum without any sample was obtained before analysing the samples of interest. Solid state UV-Vis spectroscopy of plain wool and copper wool was carried out using a Perkin-Elmer Lambda (650) UV-Vis spectrophotometer. Square pieces of wool were analysed without any further sample preparation. The light absorbance of the samples was determined by scanning the samples from 200 to 800 nm. Baseline was obtained using plain wool. The thermal gravimetric (TG) analysis of plain and copper wool was carried out using Setaram TG-92 to determine the effect of temperature on the physical properties of the catalyst. The samples were heated to 700°C at a heating rate of 5°C min^{-1} and the corresponding loss in wool weight was recorded. Scanning Electron Microscopy (SEM) images of plain wool and copper wool were obtained using a JEOL SEM 6480LV. Wool fibres were stuck onto a double sided carbon adhesive tape, which was then mounted on the sample holder. The plain wool and copper wool samples were coated with gold for improved imaging. Energy Dispersive X-Ray analysis (EDX) was carried out to determine the elemental composition of plain wool and copper wool using an Oxford INCA X-Act SDD X-ray detector coupled to the SEM6480LV. Sample preparation for EDX analysis was similar to that used for SEM without the gold coating. The copper loading on wool was determined using X-ray photoelectron spectroscopy (XPS) carried out by the Nexus facility at Newcastle University using an Axis Nova spectroscope and CasaXPS was used to analyse the spectrum. Square samples of $1cm^2$ were prepared by sticking wool fibres of uniform lengths on a double sided adhesive tape.

2.2.4 *Reaction conditions*

(a) *Nitroaldol condensation reaction in batch*

In a typical batch process, ethanol (10 ml) was added to the catalyst (0.15 g) and stirred. Benzaldehyde (1 mmol), nitromethane (10 mmol) and triethylamine (0.16 mmol) were added and reaction was carried out at 25°C for 24 hours in triplicates in a reaction carousel (Figure 2.1). Neat samples drawn out at 4, 6 and 24 hours were used for NMR spectroscopic and GC

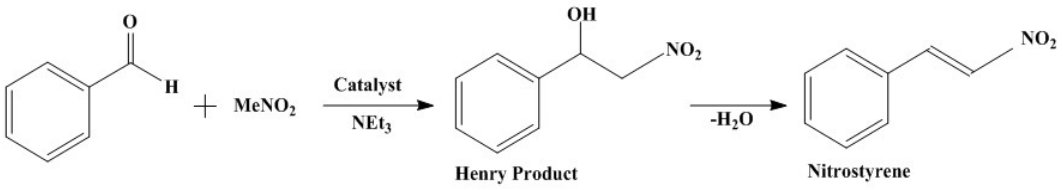


Figure 2.1: The Henry reaction between benzaldehyde and nitromethane. This also illustrates the potential side product, nitrostyrene.

analysis to determine the conversion of the reaction. The enantiomeric excess was determined using HPLC.

(b) *Nitroaldol condensation reaction in SMDR*

The SMDR primarily is a circulating batch reactor which is centrally fed overhead (Figure 2.2). The reactor consists of a rotor (Heidolph RZR 2021), with a variable speed, connected to a glass disc (12 cm diameter and 2 mm thick) with a metal rod. The disc is the critical component of the reactor as the cloth rests on top of the disc. The cloth was cut to the size of the disc and placed on the disc without any fastening. The disc is surrounded by a steel chamber shaped as a funnel. The liquid feed is pumped to the centre of the disc by a peristaltic pump (Watson-Marlow 503U) through a feed pipe. The position of the overhead feed pipe is such that the liquid feed impacts the centre of the cloth with minimum splashing to allow maximum wetting of the cloth. The centrifugal force enables the formation of uniform film of the feed on the surface of the cloth and ensures good contact between the reactants and the cloth. The steel funnel is used to contain and funnel down any liquid spun off the edge of the disc into the reactant vessel. In order to facilitate the reaction under an inert atmosphere, the entire set-up was sealed on all sides with perspex panels with gas inlet at the bottom and an outlet at the top. Before the reaction, the box housing the reactor was purged and filled with nitrogen gas under 0.3 bar pressure and 8 L min^{-1} gas flow rate. The entire box was filled with nitrogen in 15 minutes, after which the gas flowrate was reduced to 2 L min^{-1} to compensate for any gas leakage. The nitrogen atmosphere was ensured by monitoring depletion of oxygen levels using an oxygen probe.

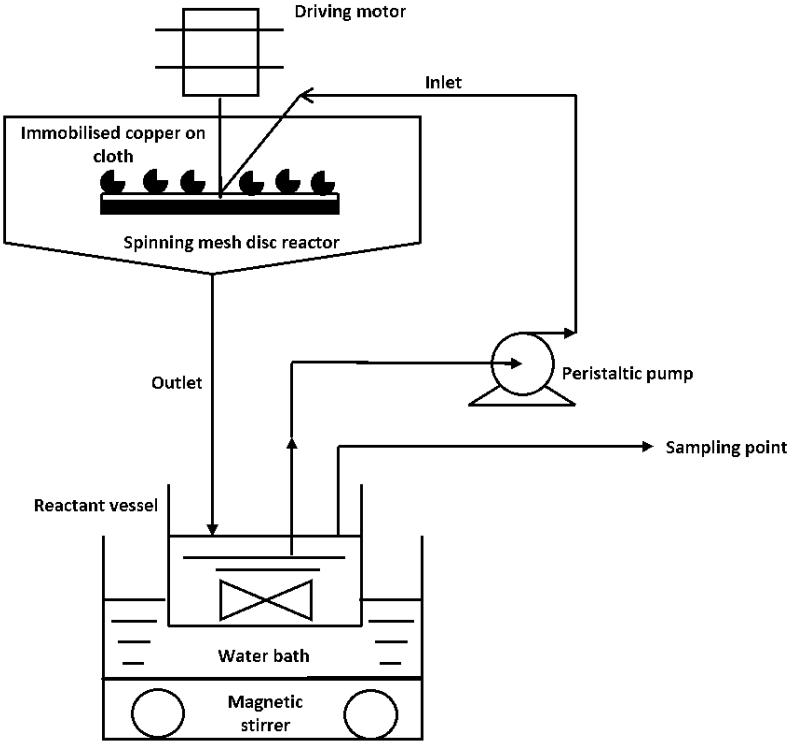


Figure 2.2: Schematic diagram of the spinning mesh disc reactor (SMDR) used in the present study

Benzaldehyde (1 mmol), nitromethane (10 mmol), triethylamine (0.16 μmol) and dodecane (0. 5 mmol) were dissolved in 250 ml ethanol and the reaction was carried out at 25°C. The disc was connected to the driving motor and spun at the desired speed. The reaction was started by turning on the peristaltic pump and was carried out for 5 hours. 1.5 ml of samples were taken out every 5 minutes for the first 30 minutes and every hour thereafter and analysed using GC and the final sample was also analysed using NMR spectroscopy. The effect of spinning speed and shear stress on the reaction conversion was studied. The surface shear is directly proportional to the spinning speed and reaches a maximum at the disc edge. The following equation is used to calculate the surface shear [23]:

$$S = \left(\frac{3QR\omega^4}{2\pi\nu^2}\right)^{1/3} \tag{2.1}$$

Where, S = shear stress (s^{-1}); Q = Volumetric flow rate ($m^3\ s^{-1}$); R = Radial distance (m); ω = Angular velocity (rad s^{-1});ν = Kinematic viscosity ($m^2\ s^{-1}$)

The average surface shear on the surface of the disc can be calculated using the following equation:

$$\bar{S} = \frac{1}{R}\int_0^R Sdr = \frac{3}{4}\left(\frac{3QR\omega^4}{2\pi\nu^2}\right)^{1/3} \tag{2.2}$$

In the present study, the average surface shear was used to characterise the performance of the reactor.

2.2.5 *Gas Chromatography (GC)*

The conversion of the reaction and the formation of the product was monitored using a Varian CP-3800 gas chromatograph model. A CP-Sil-8 CB column (30 m$\times$0.32 mm) with a film thickness of 0.25 μm was used. The injector and detector temperature were maintained at 220°C and 250°C respectively. The column oven was held at 40°C for 3 minutes, heated to 200°C at 10°C min^{-1} and held for 2 minutes thereafter. Dodecane (0.6 mmol) was used as the internal standard (IS).

2.2.6 *Proton NMR Spectroscopy*

Detection of product and conversion of the reaction was determined by analysis of the ^{1}H NMR spectra. Neat samples were used for all the analysis. 0.1 ml of the sample was dissolved in 0.6 ml of deuterated chloroform and the samples were run in a 300 MHz Bruker Spectrometer. Conversion using NMR spectroscopy was determined by the analysis of the ^{1}H integral of benzaldehyde at 9.94 ppm to the ^{1}H integral of PhCH(OH)CH$_2$NO$_2$ at 5.45 ppm, as previously detailed elsewhere [23].

2.2.7 *High Performance Liquid Chromatography (HPLC)*

To determine the enantiomeric excess of the product, HPLC was carried out using Agilent technologies 1260 Infinity with UV detector (254 nm). An Astec Chirobiotic V2 chiral column was used. The flow rate of IPA and hexane (1:9) was maintained at 0.5 ml min^{-1}. The enantiomeric excess (ee) was calculated using the following equation:

$$ee = \frac{(R-S)}{(R+S)} \tag{2.3}$$

2.3 Results and discussion

2.3.1 *Spectroscopic analysis of wool*

FTIR spectra analysis was used to determine the functional groups present in wool (Figure 2.3). In plain wool, the broad peak at 3280 cm^{-1} can be attributed to N-H stretching bond and the sharp peaks at 1633 cm^{-1}, 1525 cm^{-1} and 1236 cm^{-1} is due to the presence of S-H and N-H groups present in wool. Post PEI treatment, the new peak at 3070 cm^{-1} and 1392 cm^{-1} is due to the N-H stretching bond induced by PEI. Along with the reduced intensity of the peak at 2929 cm^{-1}, it can be inferred that PEI was successfully immobilised on wool [9]. In the FTIR spectra of copper wool, there is a reduction in the intensity of peaks at 1523 cm^{-1}, 1639 cm^{-1} and 1234 cm^{-1} indicating the interaction of the metal with the amide I (N-H stretching bond), amide II (C-N stretching bond) and amide III (N-H bending bond) indicating the formation of copper-keratin

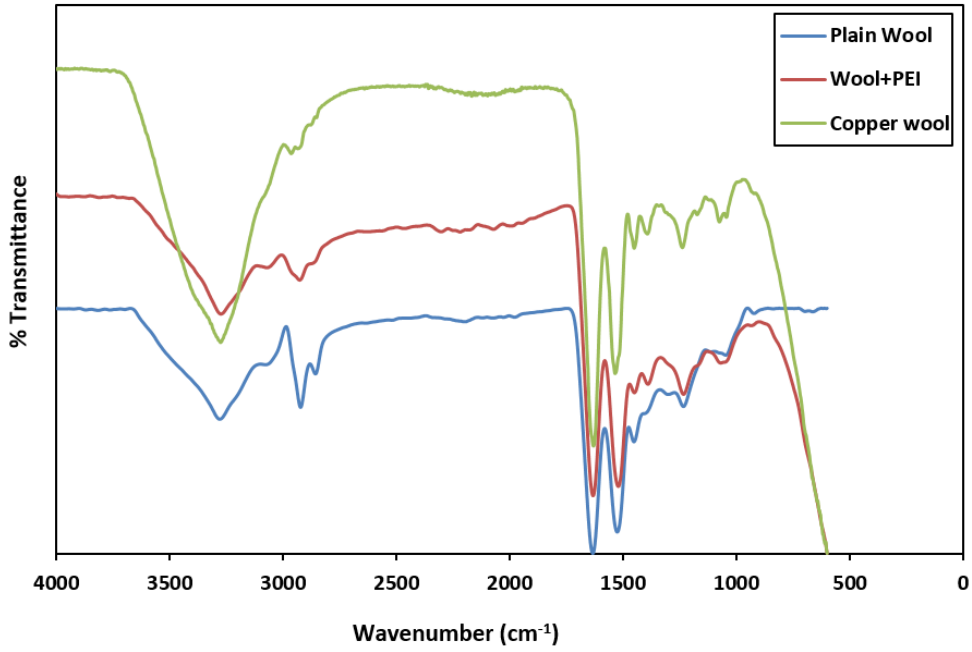
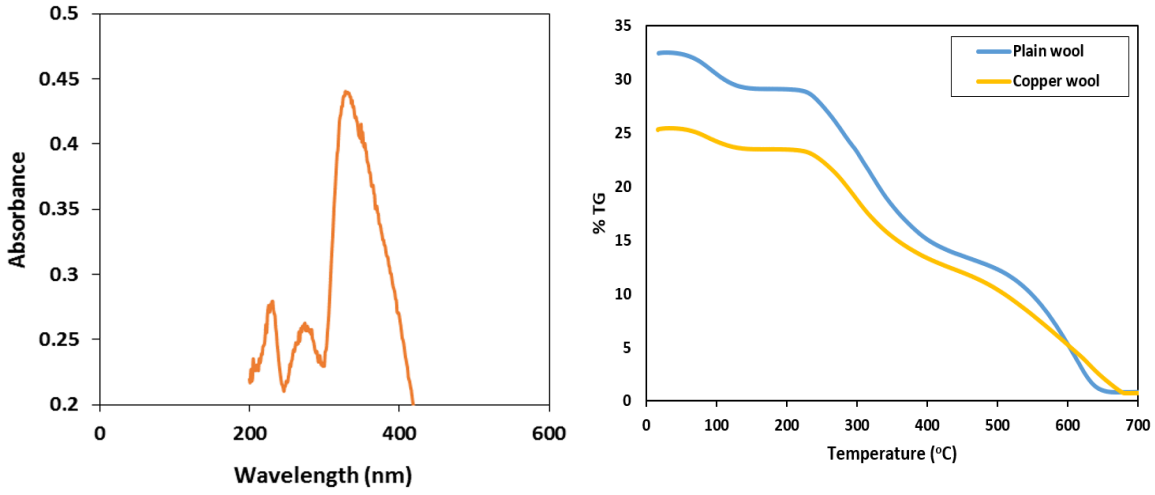


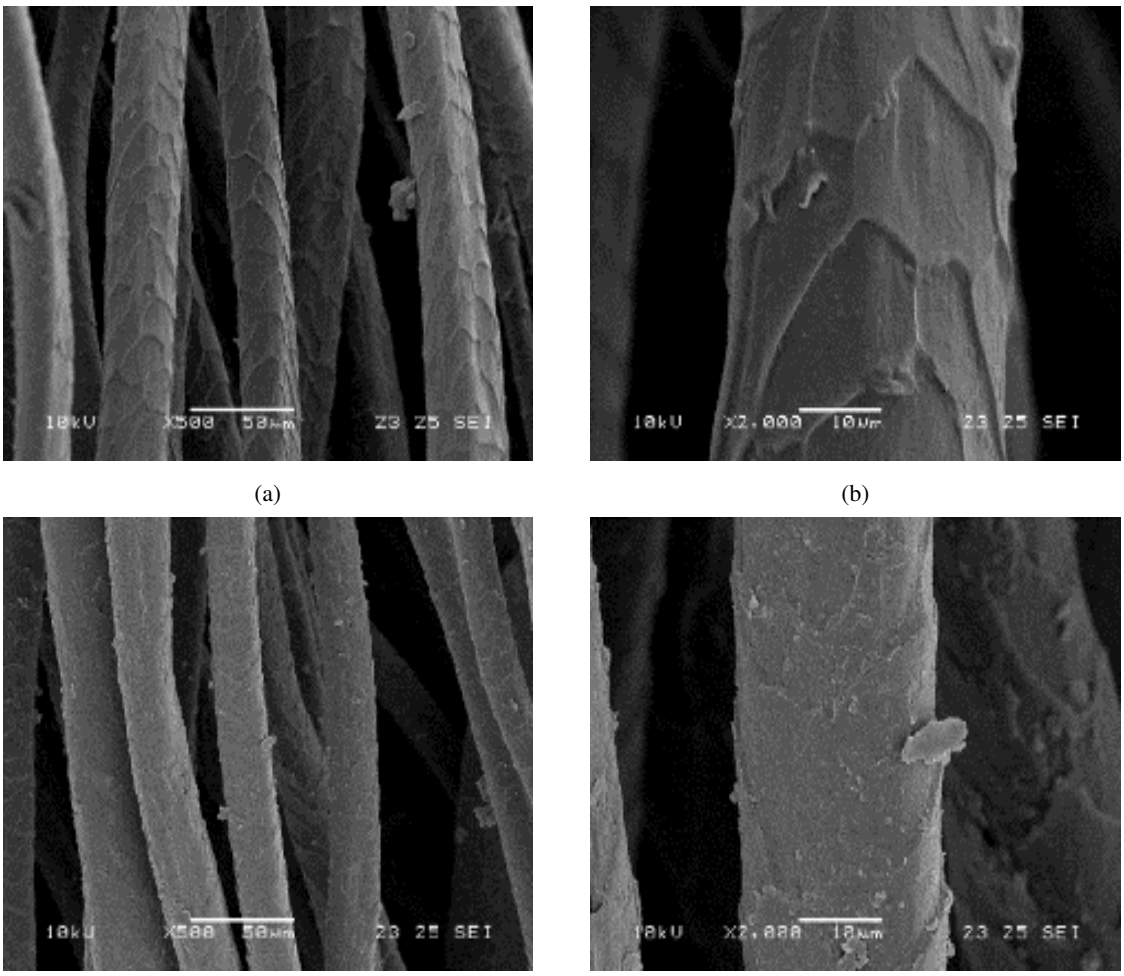
Figure 2.3: FTIR spectrum of plain wool and copper wool



(a) (b)

Figure 2.4: (a) Solid state UV spectrum of plain wool and copper wool and (b) TG analysis of plain wool and copper wool in air

complex on wool [24]. The immobilisation of copper on wool was further confirmed by UV spectroscopy. Post copper immobilisation on wool, a broad peak was observed at a wavelength of 336 nm (Figure2.4(a)). Copper has a high affinity for nitrogen base groups and thus, we hypothesise that the Cu(II) ions are ligated to the nitrogen containing amino acid residues present in wool [25]. The thermal stability of plain and copper wool was carried out by TG analysis as shown in (Figure 2.4(b)). In the TG profile of plain wool, the initial weight loss up to 120°C is due to the hydrogen



(a) (b)

(c) (d)

Figure 2.5: SEM images of: (a-b) Plain wool, (c-d) Copper wool

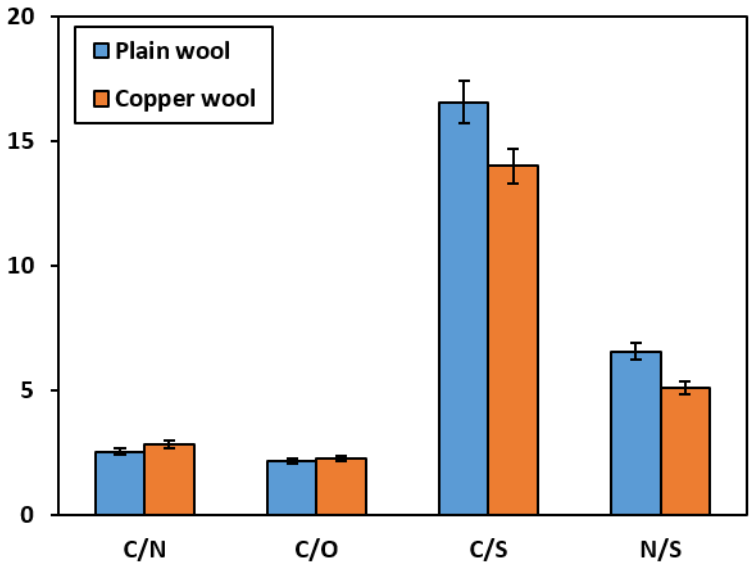


Figure 2.6: Elemental ratios present in plain wool and copper wool analysed using EDX

bonded water within the wool fibres. The second step of weight loss occurs at over 200°C due to the thermal decomposition of cysteine and terminal amino acid groups. From 430°C to 600°C, the weight loss is due to the oxidation of the carbonised residue. In the TG profile of copper wool, an increase in the weight loss in the third stage of decomposition compared to plain wool confirms the immobilisation of copper on wool [26]. SEM analysis of wool was carried out to understand the morphology of plain and copper wool. The morphology of plain wool fibres is characterised by tiled cuticle cells on the surface of the fibre with a fibre diameter of 2.6 μm (Figure 2.5 (a-b)). Individual copper particles are not clearly visible in the SEM image of copper wool, however, the loss of definition of the scales on the fibre surface is indicative of a coating on top of them resulting in surface modification of wool (Figure 2.5 (c-d)). The results from EDX analysis of wool are expressed as elemental ratio as quantification of individual elements is associated with instrument inaccuracy (Figure 2.6). A copper loading (determined via EDX) of 0.82% was obtained on the surface of wool. The change in C/N post copper immobilisation is almost negligible indicating that none of the surface lipids were lost from the wool cloth. Wool remains un-oxidised post copper immobilisation as C/O is almost constant [27]. A decrease in C/S and N/S post copper immobilisation may be ascribed to the presence of sulphur from copper triflate which further confirms copper immobilisation on wool. The survey spectrum obtained from the XPS analysis (Appendix A) of plain wool indicated the presence of carbon (82.55 %), oxygen (10.05 %), nitrogen (5.7 %) and sulphur (1.71 %). Immobilisation of copper triflate on wool was confirmed by the presence of copper (0.67 %) and fluorine (5.3 %). From the elemental scans, there is a clear indication of a change in the binding energy of copper wool which indicates the interaction between the amino acid residues present in wool and copper triflate (Figure 2.7(a-d)). Also, the presence of Cu2p in copper wool further confirms copper immobilisation on wool (Figure 2.7(e)).

2.3.2 *Copper cloth for nitroaldol condensation reaction*

The reaction in batch was carried out using copper triflate immobilised on wool and free copper triflate and a reaction conversion of 85% and 57% was obtained respectively (Table 2.1). Catalyst immobilisation is usually associated with a reduction in reaction conversion compared to the free catalyst. The reactants in the bulk solutions have to overcome the diffusion resistance offered by a stagnant liquid film on the surface of the cloth to come in contact with the catalyst present on and within the cloth. However, in the present study, the increase in conversion may be due to the formation of copper-protein complex on wool which maybe a more efficient catalyst. In the SMDR, the conversion of the reaction was found to be 90% accompanied by a reduction in the reaction time to 5 hours (Figure 2.8(a)). The conversion obtained in the present study is higher than that reported in literature for Henry reaction in batch [23]. A higher reaction conversion in the SMDR is due to the decrease in mass transfer resistance between the catalyst and the reactant

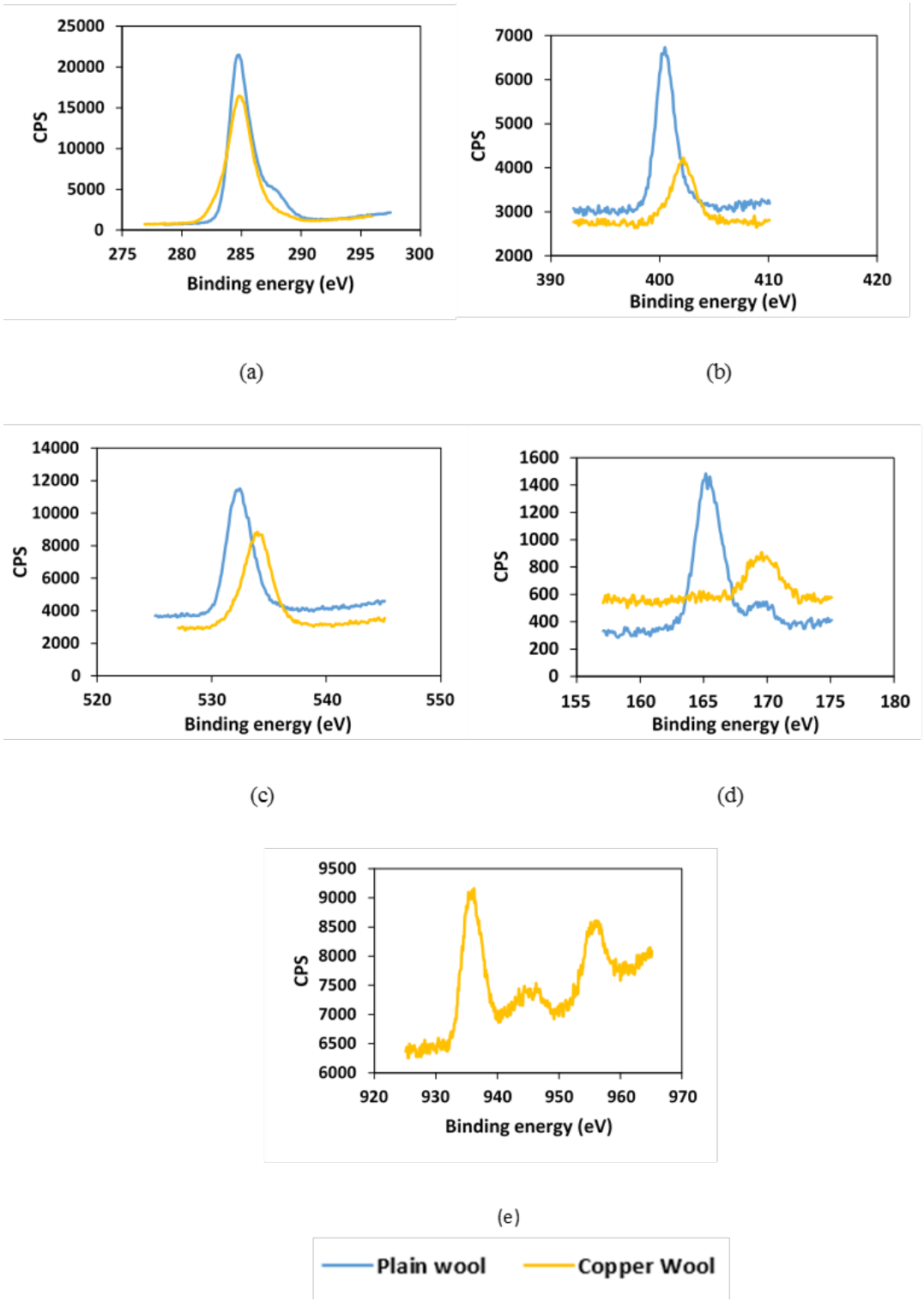


Figure 2.7: Elemental scans of plain wool and copper wool from XPS: (a) C1s, (b) N1s, (c) O1s, (d) S2p and (e) Cu2p

in the thin film on and within the cloth owing to the rapid mixing associated with the spinning disc as opposed to the liquid flow in a batch system which is centrifugally forced over the immobilised catalyst. Another reason for this can be due to the increased residence time of the reactant on the immobilised catalyst on the cloth surface ensuring a constant presence of the reactant in reaction

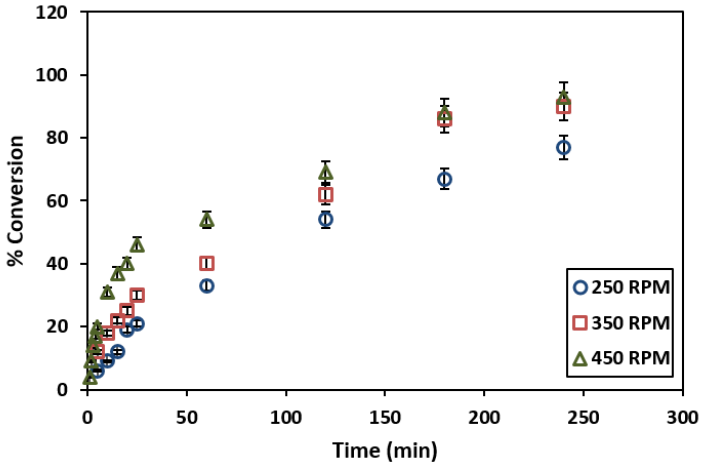
Table 2.1: Results of nitroaldol condensation reaction in batch using free and immobilised copper triflate

Catalyst	**Time(h)**	**Conversion(%)**
Copper triflate (homogeneous, batch)	4	38
	6	42
	24	57
Copper cloth (heterogeneous, batch)	4	41
	6	63
	24	85

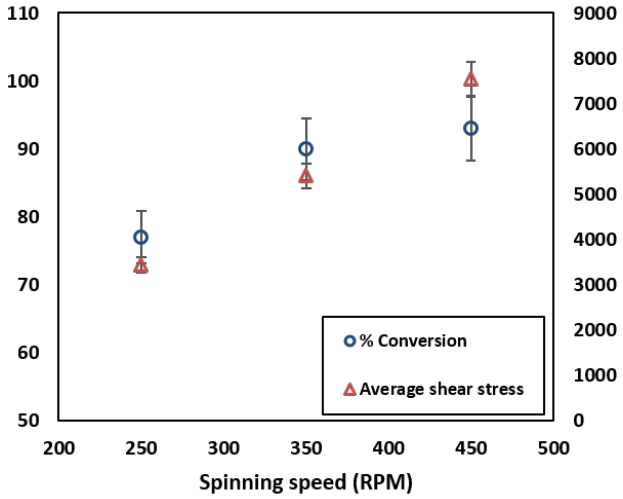
zone. The effect of spinning speed and feed flowrate on reaction conversion was studied. Average surface shear is proportional to the spinning speed of the disc and research has shown that this results in a higher degree of mixing on the surface of the disc, hence improving the overall reaction conversion [9, 10]. The reaction conversion increased from 77% to 93% as the spinning speed of the disc increased from 250 to 450 RPM at a flowrate of 3 ml s^{-1} (Figure 2.8(b)). A higher flowrate of the reactant feed results in an increase in conversion as the frequency of contact between the immobilised catalyst and the reactant is much higher. There was a 4% increase in the reaction conversion on an average as the flowrate increased from 3 ml s^{-1} to 5 ml s^{-1} (Figure 2.8(c)). Enantiomeric studies were carried out and the 'ee' of the reaction catalysed by copper wool was found to be 64% (SMDR), compared to essentially a racemic mixture obtained by reaction catalysed by free copper triflate.

2.3.3 *Catalytic activity of wool for nitroaldol condensation reaction*

As a part of the continuing studies of the reaction, it was found that plain wool exhibited catalytic properties by itself. A conversion of 77% was observed with plain wool after 24 hours in batch compared to 85% conversion obtained with copper wool under similar experimental conditions (Table 2.2). Wool is made of natural amino acids to which its catalytic activity can be attributed. The aldol reaction has been shown to be catalysed by amino-acids, an example being proline [28] and other metal free systems are well known [29]. Post reaction, the wool acquired a yellowish-brown tint which may be due to the oxidation of tyrosine to quinone, as tyrosine is highly reactive and influences the property of wool [30]. In the SMDR, reaction conversion increased with increase in the spin speed of the disc (Figure 2.9). Also, this allows the liquid feed to flow through the cloth, increasing the contact with the functional groups present in wool. A



(a)



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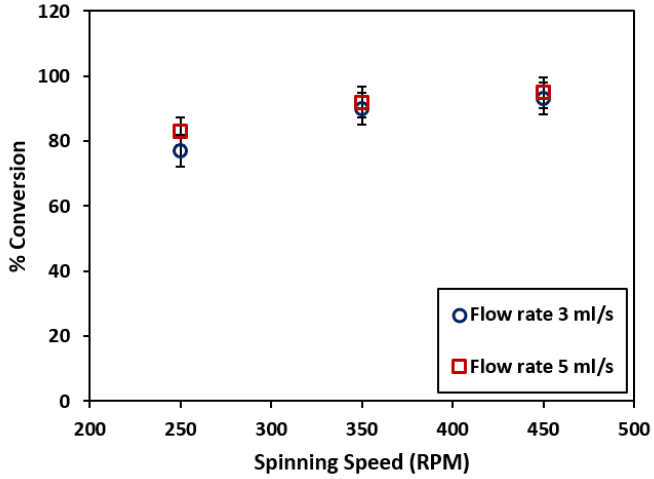


(c)

Figure 2.8: (a) Effect of spinning speed on reaction conversion and (b) Correlation between reaction conversion and average surface shear (Flow rate: 3 ml s^{-1} and spinning speed 350 RPM) and (c) Effect of feed flowrate on reaction conversion

Table 2.2: Results of nitroaldol condensation reaction in batch catalysed by copper triflate and plain wool

Catalyst	Time(h)	Conversion(%)
Copper triflate (homogeneous, batch)	4	38
	6	42
	24	57
Plain wool (heterogeneous, batch)	4	35
	6	50
	24	77

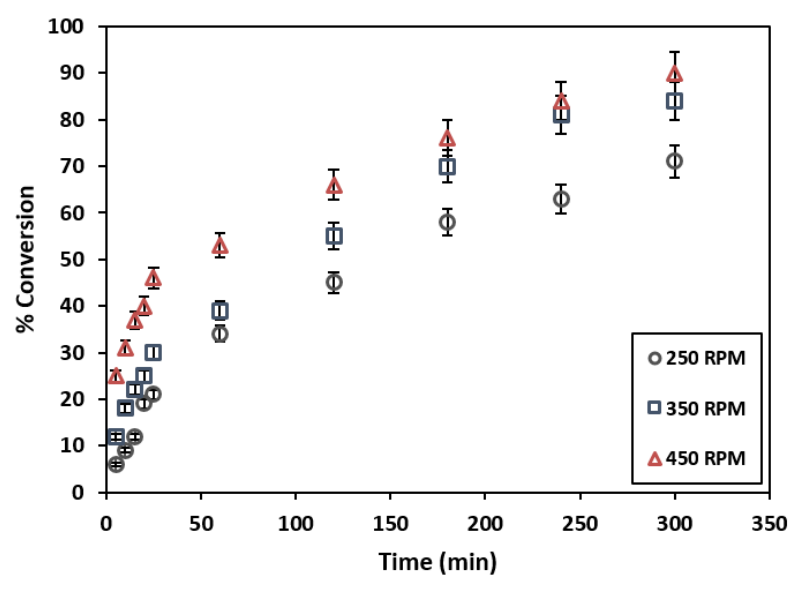
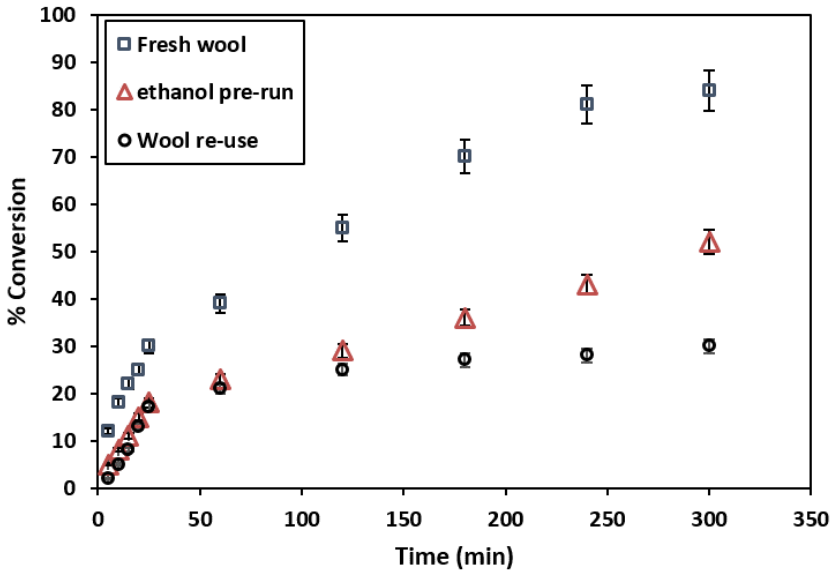


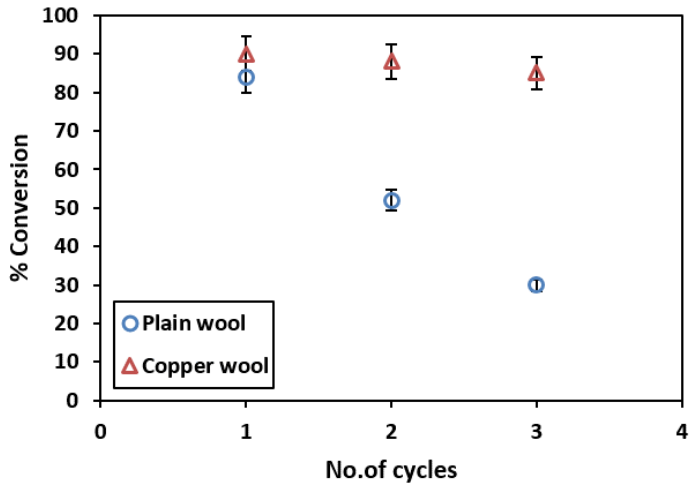
Figure 2.9: Effect of spinning speed on reaction conversion catalysed by wool. Flow rate: 3 ml s^{-1}

maximum conversion of 85% was obtained at a spin speed of 450 RPM [25].

The re-usability of plain wool as a catalyst was tested by measuring the product formation using the same cloth for 2nd cycle, in the SMDR. The reaction conversion decreased by almost 40% on re-use of wool (Figure 2.10(a)). Comparatively, there was only 3% reduction in conversion on re-use of copper wool over 3 cycles under similar experimental conditions (Figure 2.10(b)), which further confirms the stability of the copper-wool complex formed. This demonstrates the robustness of the copper cloth for multiple cycles. To study the effect of solvent on plain wool, fresh wool was treated with ethanol for 4 hours in the reactor under identical reaction conditions



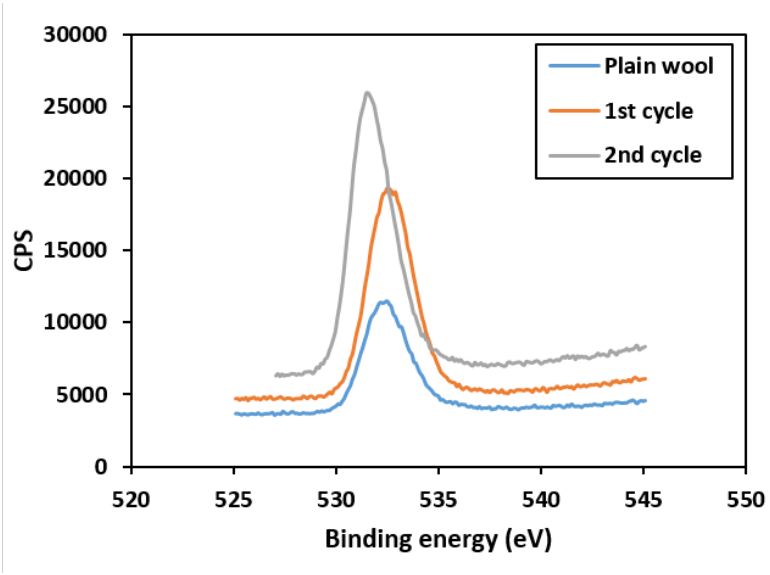
(a)



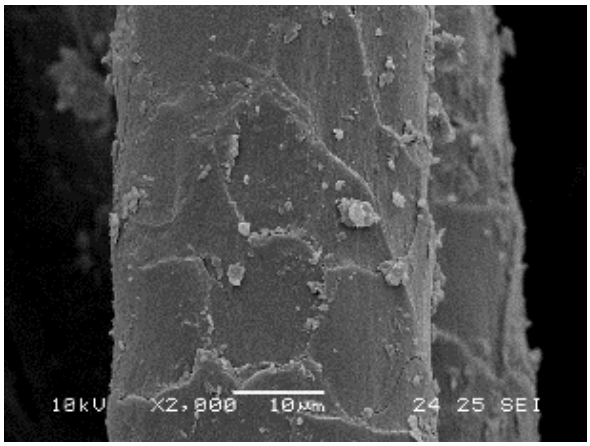
(b)

Figure 2.10: (a) Effect of wool re-use on reaction conversion and (b) Comparison of re-use of plain wool and copper wool over 3 cycles. (Flow rate: 3 ml s^{-1}, spinning speed: 350 RPM)

and the solvent treated wool was used for Henry's reaction. It was found that conversion was closer to that obtained by carrying out the reaction with used wool. The binding energy of wool when re-used increases, as the O1s scan indicates the possible oxidation of wool accompanied by a change in the fibre morphology (Figure 2.11 (a-b)). The carboxylic acid groups present in wool are active reaction sites and easily accessible to alcohols of lower molecular weight [30]. Esterification of carboxylic acid functionality can occur in the presence of ethanol, which could be the reason for decreased product formation of wool on 2^{nd} run. Additionally, the reduction in conversion may also be due to the high surface shear on the disc surface which may cause leaching of proteins into the solution, thus reducing the amount of catalyst sites available for reaction.



(a)



(b)

Figure 2.11: (a) O1s scan of used wool and (b) SEM image of used wool

2.4 Conclusion

In this study, copper triflate was successfully immobilised on wool using a simple protocol. The copper cloth and free copper triflate were used as a catalyst for Henry reaction in batch and a conversion of 85% and 57% were obtained respectively after 24 hours proving that the copper-protein complex was a better catalyst. The reaction was carried out in the SMDR with the copper cloth and a conversion of 90% was achieved after 5 hours at a spinning speed of 350 RPM, accompanied by an increase in 'ee' to 64%. The marked increase in the reaction conversion is due to the improved mass transfer on the surface of the disc. The reaction conversion increased by an average of 4% as the feed flow rate increased from 3 ml s^{-1} to 5 ml s^{-1}. There was a steady increase in the conversion with an increase in the spinning speed from 250 RPM to 350 RPM accompanied by the increase in the average surface shear and better mixing on the disc surface. In addition to being a good catalyst support, wool also exhibited catalytic properties on par with conventional

metal catalysts. A maximum conversion of 85% was obtained at a spinning speed of 450 RPM in the SMDR. However, there was a reduction in reaction conversion on reuse which may be due to the oxidation of wool or change in the fibre morphology. This shows that wool is a potential green catalyst for chemical processes if the catalyst deactivation can be controlled. Overall, the reactor was successfully used for the Henry reaction and can be used for other chemical processes which can benefit from improved reaction engineering. Work is on-going to investigate this process for other reactions and future work will aim to examine the economic and life cycle assessment of this technology.

Acknowledgement

The authors thank the University of Bath for the PhD scholarship of PS, Department of Chemical Engineering, Department of Chemistry for the technical support and EPSRC for funding the XPS analysis at University of Newcastle. The project is also funded by European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no FP7- 333952 (SMDR).

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Chapter 3

Process intensification of immobilised enzymatic reactors

The previous chapter reported process intensification of bench scale chemo catalysed organic reaction using the SMDR. Another application where process intensification is receiving increased research interest is enzymatic reactions. Enzymes are popular catalysts for reactions where high product selectivity is necessary [1]. Enzymes immobilised on a suitable substrate are preferred over free enzymes as they offer better resilience to the harsh reaction conditions and can be easily separated from the reaction media [2]. Conventional reactors for immobilised enzyme catalysis are limited by mass transfer resulting in longer times and hence greater energy costs. Scale-up is also a challenge as it requires a large amount of expensive enzyme catalyst [3].

Process intensified reactors are a promising alternative to facilitate immobilised enzyme catalysis. This chapter provides a detailed review of the recent advances in process intensified enzymatic reactors (PI-ERs) using both free and immobilised enzymes. The chapter also addresses the limitations of the conventional reactors for enzymatic reactions and the future scope for PI-ERs for applications in pharmaceutical and fine chemical industries.

<table>
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<tr><td colspan="10">Process Intensification of Immobilised Enzyme Reactors</td></tr>
<tr><td colspan="10">Publication status (tick one)</td></tr>
<tr><td>draft manuscript</td><td></td><td>Submitted</td><td></td><td>In review</td><td></td><td>Accepted</td><td></td><td>Published</td><td>✓</td></tr>
<tr><td>Publication details (reference)</td><td colspan="9">Shivaprasad, P., & Carolina Emanuelsson, E. A. (2018). CHAPTER 11: Process Intensification of Immobilized Enzyme Reactors. In RSC Green Chemistry (Vol. 2018-January, pp. 249–267). Royal Society of Chemistry.</td></tr>
<tr><td>Candidate's contribution to the paper (detailed, and also given as a percentage).</td><td colspan="9">Formulation of ideas (60%): Emma Emanuelsson suggested this review and provided an outline for the chapter. I contributed to carrying out the literature review to identify the recent advances in enzymatic synthesis using PI reactors.

Design of methodology: Not applicable as this chapter is a review article.

Experimental work: Not applicable as this chapter is a review article.

Presentation of data in journal format (75%): I prepared the first draft of the review and the co-author provided extensive feedback to the subsequent drafts. I was responsible for incorporating comments from the book editors and publishers towards the end.</td></tr>
<tr><td>Statement from Candidate</td><td colspan="9">This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.</td></tr>
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3.1 Introduction

Enzymes belong to the protein family and can catalyse chemical and biochemical transformations that take place at room temperature and pressure [1]. Enzyme catalysed reactions have found a number of applications in the food and pharmaceutical sectors due to their biocompatibility with the human body. More recent application of enzyme catalysis includes synthesis of intermediate scale chemicals [4–6] and production of polymers [7–9]. However, the reactions have only been carried out on a bench scale and industrial application has been limited by a decrease in reaction yield and selectivity during scale-up [10, 11].

One way towards a more sustainable future is to change the current unsustainable practices in process industries. Process intensification (PI) can reduce process inefficiency [12] by transforming the current engineering practices to make it more sustainable and environmentally benign without hampering production efficiency and profitability [10]. PI can be achieved in the form of novel/hybrid reactor design or by novel processing routes. The underlying features of these reactors include intensification of mass and heat transfer accompanied by minimum downstream processing. The aim of this chapter is to introduce a range of new reactors that have been developed since the early 90's. These reactors have been used to improve existing reaction mechanisms and also to enable scale-up of more novel applications like enzyme catalysed reactions. Other chapters in this book (see chapters 9 and 12) deal with alternate energy source for enzyme reactors like microwave and ultrasonic energy to achieve PI.

This chapter will firstly provide an overview of enzyme immobilization and conventional reactors with their shortcomings. Thereafter, process intensification of reactors with a focus on immobilized enzymes will be discussed although some of the reactors support both free and immobilized enzyme reactions. This chapter will conclude with a perspective on the future opportunities for process intensified enzyme reactors.

3.2 Enzymes as catalysts

Enzymes are natural catalysts that have gained importance for improving chemical reaction rates and have the potential to surpass the catalytic activity of inorganic catalysts. They are highly specific catalysts operating under mild reaction conditions and their chirality enables them to be employed for enantioselective synthesis in pharmaceutical industry, where end products of high optical purity are desirable. Reactions catalysed by free enzymes follow a simple operation protocol; however, the key drawbacks with free enzymes are the deactivation under harsh operating conditions and the additional separation step at the end of the reaction. Immobilization of enzymes broadens enzyme applicability and mitigates the issue of instability [13] due to the increased me-

chanical and thermal stability provided by the support material, which also facilitates enzyme recovery and reuse. Enzyme immobilization methods can be broadly classified as below (Figure 3.1). An in depth analysis of immobilization techniques is beyond the scope of this chapter,

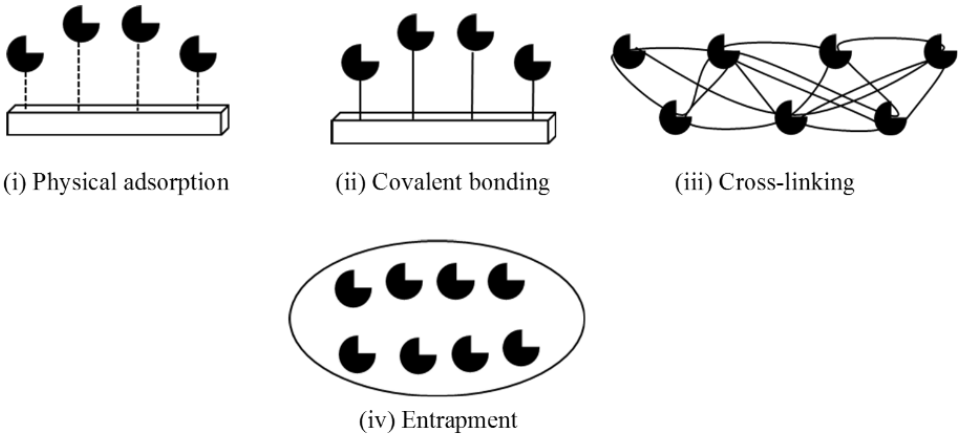

Figure 3.1: Classification of enzyme immobilization methods

and has been published by the author [3]. Briefly, enzyme immobilization can either be physical or covalent in nature. Physical binding of enzymes involve ionic, van der Waal's or hydrophobic interactions with the support surface. The method is simple and cost-effective, but enzyme leaching and instability in the reaction environment is a key drawback [2]. Covalent binding of enzymes provides better enzyme stability and greater re-usability in both aqueous and organic media. Chemical modification of the enzyme during the process of immobilization is one of the main disadvantages [14]. Enzyme entrapment within a polymeric network or sol-gel is another immobilization technique, where the enzyme is safeguarded against mechanical shear and hydrophobic solvents. Increased resistance to mass transfer and low enzyme loading are some of the drawbacks of this method [15]. Carrier free immobilization is possible by using a cross-linking agent like gluteraldehyde, which binds the enzymes to each other without the need for a support. Reports of higher enzyme stability and activity have been reported for cross-linked enzymes. However, some of the cross-linking agents are specific for certain enzymes and it is necessary to optimize the amount of crosslinking agent as an excess can adversely affect the enzyme activity and productivity [16].

3.3 Enzymatic reactors: Conventional vs Process Intensified Reactors

This section will focus on the most commonly used enzyme reactors including batch, packed bed and fluidized bed reactors and some of the short comings, which have led to the need for process

intensification in bioprocessing.

3.3.1 *Conventional reactors and Enzyme Catalysis*

Batch stirred reactors (BSTR) used to be the popular choice for enzymatic reactions. These are useful to test novel reactions with low reaction volumes and they have been employed for both free and immobilized enzyme catalysis [17, 18]. However, there are inherent problems like enzyme separation and recovery from the reaction medium, low productivity due to the time consuming operations of filling, emptying and cleaning the reactor between batches [19].

Another commonly used reactor for large scale enzyme catalysis is the packed bed reactor (PBR), in which the enzyme is immobilized on a support, which is packed in a column or a pipe. The PBR provides a large interfacial area, easy catalyst recovery and re-usability due to using immobilized enzyme and the structured enzyme packing allows for excellent contact between the enzymes and the substrate in comparison to stirred tank reactors. An additional advantage is the elimination of by-products to a large extent [20]. Successful applications of PBR include production of biodiesel [21], lipid hydrolysis [22] and acidolysis of oil [23]. The key disadvantage is the increase in pressure drop, especially for small sized packings. Also, the availability of enzyme per unit volume in the reactor is reduced with an increasing packing size, thereby, reducing the efficiency of the reactor volume [24].

The fluidized bed reactor (FBR) is a form of packed bed reactor, but the catalyst is in a fluidized state due to the counter-current contact between the packing and the fluid phase [11]. Continuous agitation of the solid and the liquid phase ensures a uniform temperature distribution, but may lead to catalyst leaching and agglomeration. These reactors have been used less in comparison to PBRs for enzyme catalysed reactions. A higher conversion and a lower pressure drop for the enzymatic hydrolysis of oil was found in the FBR in comparison to the PBR [25], and the interesterification of oils, was carried out successfully in the FBR but not in the PBR due to a higher pressure drop [26]. The main disadvantage of the FBR is the need for large packing size reducing the enzyme loading per unit volume and the overall efficiency of the reactor as well as an increased risk of bypassing and channelling of both liquid and gas phases [27].

To summarize, though conventional reactors have been successfully used for enzymatic reactions to produce the desired product, problems associated with scale-up, product purification and mass transfer limitation in particular, have been the driving force for identifying alternate reactor design to effect process intensification.

3.3.2 *Process Intensified Enzymatic Reactors (PI-ER)*

Process Intensification (PI) can achieve the control of chemical reaction on a molecular scale and the advantages are: (i) enhanced reaction rate with a reduction in the size of process equipment, (ii) increased selectivity of the product resulting in waste reduction, (iii) easy product separation, which otherwise is responsible for major energy consumption, (iv) manufacturing process for novel and advanced products can be better tailored [28]. PI can be achieved either by modification of the reactor design or by changing the operating parameters used. Modification of existing reactor design has led to a new class of hybrid reactors incorporating membranes, monoliths, microchannels and rotating discs and these will be focused in this chapter. These reactors have the potential for enhancing the overall reaction rate by increasing the effective transport rates and/or by providing multi-functionality to devices for process, which can benefit from improved reaction engineering [29]. The aim of this chapter is to highlight the recent developments in the field of PI-ER. Among the different PI-ERs, enzymatic membrane reactors (3.3.2.1) and microreactors (3.3.2.2) are well researched while monolithic (3.3.2.3), rotating packed bed (3.3.2.4) and spinning mesh disc reactors (3.3.2.5) are novel classes of reactors for bioprocessing.

3.3.2.1 Enzymatic Membrane Reactor (EMR)

Enzyme Membrane Reactors (EMR) have gained popularity for catalysing bioconversions since the first enzyme immobilization took place in the 1950s. There are two ways in which the enzymes can be incorporated in the EMR: (i) suspension in the solution which is in turn compartmentalized by the membrane (Figure 3.2a) and (ii) immobilization within the membrane matrix (Figure 3.2b). The first configuration is analogous to an integrated stirred tank reactor - membrane separation unit, while in the second configuration, the membrane functions both as a catalyst support and as a separation medium [30].

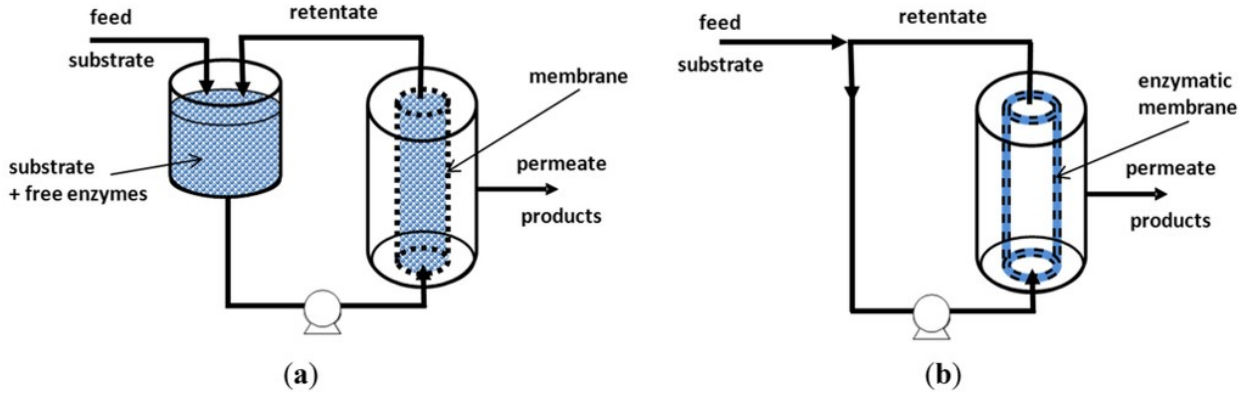

Figure 3.2: (a) Free enzyme membrane module and (b) Membrane immobilised with enzyme module. Adapted from [31]

The driving force in these reactors can be pressure, electric or chemical potential. EMR has the potential to circumvent the disadvantages of conventional enzyme reactors due to the following attributes:

- EMRs allow for continuous mode of operation and is supported for both free and immobilized enzyme reactions [32].
- The combination of chemical reactions accompanied by in-situ product separation result in an increased reaction conversion. This also simplifies enzyme recovery and reuse [33].
- The selectivity of the reaction can be improved and tailored by optimizing the enzyme-membrane combination [34].
- The moderate operating conditions ensure enzyme activity is not lost due to high temperature and pressure [32].
- EMRs are attractive for enzymatic reactions on an industrial scale as they are a greener alternative to the existing technologies in terms of energy saving and process waste reduction ensuring production of end products with high purity [35].

The selection of the membrane depends on the nature and size of substrate, enzyme and product(s). Ultrafiltration membranes are mostly used for reactors employing free enzymes as their pore size range (1 to 100 nm) is sufficient to retain a wide range of enzymes and the unique asymmetric pore size distribution along the length of their surface allows for higher flow rate of the permeate, reducing clogging and hence, allowing for easy cleaning of the membrane[36, 37]. The enzyme stability on a particular membrane is critical, and the choice of membrane material is usually made based on membrane characteristics, like pore size distribution, molecular weight cut off, operating pH, temperature and pressure and resistance to chemicals [36, 38]. Ceramic membranes are generally preferred to polymeric membranes as they provide greater chemical resistance and can operate at higher temperatures and pressures although the uptake of polymeric membranes is increasing and there is a lot of research focusing on improving the operating envelope of polymeric membranes [37, 39].

EMRs are conventionally classified based on the reactor configuration and the hydrodynamics of the system, into CSTR and plug flow EMR. However, with the advent of multi-phase reactor configurations and different membrane configurations, more recently they have been classified as direct contact, diffusion and multiphase EMRs [40].

(i) *Direct contact membrane reactors*

In this reactor configuration, the substrates are introduced on the enzyme side of the reactor,

where the enzyme is either free or immobilized. Further classification of this configuration include dead end, recycle and dialysis reactors. In the dead end cell reactor configuration, both separation and reaction take place in a single compartment and pressure is used as the driving force to cause the permeation of the reactant media across the membrane. The mode of operation of this configuration is analogous to a stirred tank reactor, where the membrane divides the permeate and the retentate stream. Despite shortcomings such as low flux and membrane surface area, this configuration is popular for lab scale processes as a consequence of operation simplicity [41, 42]. In the recycle reactor configuration, the solution containing substrate and the enzyme are recycled between the reaction vessel and the ultrafiltration membrane unit, present in a closed loop. In dialysis reactors, enzyme and substrate is introduced on the same side of the membrane and the product(s) diffuse across the membrane because of the concentration gradient. Tubular and hollow fibre membranes are the commonly used membrane modules for the reactor configuration in general for applications like hydrolysis of lipids and proteins and enantioselective synthesis of protein [43–46].

(ii) *Diffusion membrane reactors*

In this class of reactor configuration, the enzyme and substrate are separated by the membrane, and the substrate diffuses through the membrane enabling the reaction. The products are then recycled back to the substrate side after reaction. Hollow-fibre modules with the enzyme placed in the shell side is usually used for this configuration [40]. Only substrates with low molecular weight can be processed, and since diffusion of the substrates into the permeate side is the rate limiting step, reaction rates are lower in this configuration [30]. This configuration has mainly been used for enzymatic conversion of sugars [47] and synthesis of proteins [48]. The limited applicability of this configuration is one of the main drawbacks.

(iii) *Multiphase enzyme reactors*

The final configuration is characterized by the interfacial contact between the enzyme and the substrate at the membrane site. The membrane separates the polar and the apolar phases and in some cases, slight pressure is applied to facilitate the phase separation. The flow can either be single pass or recycled through external vessels. This configuration is used when the enzyme is triggered by interfacial activation [43]. The applications of this reactor configuration include oil hydrolysis [49–51] and synthesis of fatty acids [52–55]. Phase mixing is one of the key disadvantages of this configuration.

EMRs have been applied for the production of pharmaceuticals [56–58] achieving high enantiomeric excess ('ee'). In the food sector [30, 59], EMRs have been used for clarifying fruit juices and lactose reduction in dairy products. Enzymatic hydrolysis of lipids is another successful appli-

cation of EMRs [50, 60, 61]. In general, immobilized enzymes have a higher stability compared to when the reactor was operated with free enzymes. An increase in yield has been achieved by increasing the enzyme loading on the membrane. Furthermore, in EMRs that incorporate nanofiber membranes, the transmembrane pressure effect on conversion and energy consumption was eliminated.

Though membrane reactors have been used in many successful transformations, EMRs still have a range of shortcomings [32] such as a loss in the enzymatic activity due to immobilization or the reaction environment and membrane fouling. Future research should focus on making advances in reactor design and control mechanism for reactor scale-up and improving the reactor efficiency by carrying out cascade and synergistic reactions and reducing fouling, for example by using tuneable membranes. EMR technology can also be extended to reactions with insoluble products to explore the potential of the UF membrane to retain solid particles [40].

3.3.2.2 Enzymatic Microreactors

Microreactors are a type of reactors that comprise of microchannels of diameter between 10-500 μm, which reduce transfer limitations and enable a greater exchange area for reactant molecules (Figure 3.3). This in turn improves mixing and heat exchange within the microchannels resulting in higher mass and heat transfer [62], resulting in fast reactions with residence times in the order of milliseconds using small amounts of enzyme and substrate for the reaction. Microreactors have been well developed for analytic and diagnostic applications [37, 63–65] and the opportunities for bioprocessing are gaining attention due to the following PI advantages:

- Enhanced reaction selectivity can be obtained using microreactors as energy can be supplied in the right form and in the right amount; thus, achieving a uniform reaction experience for all the reactant molecules [28].
- In addition to providing superior heat transfer, free radicals are terminated when they come in contact with the microchannel walls, stabilising their propagation rate [66].
- Miniaturization of the reactor reduces the energy consumption, waste generation and chemical inventory as the reaction volumes are reduced ensuring that the reactor volume is fully utilized [67].
- Scale up can easily be achieved through the replication and numbering up of each reactor unit, and thus, reducing the cost associated with re-design which facilitates faster commercial scale production [65].
- Allows for fast screening of small molecules for drug discovery for the pharmaceutical

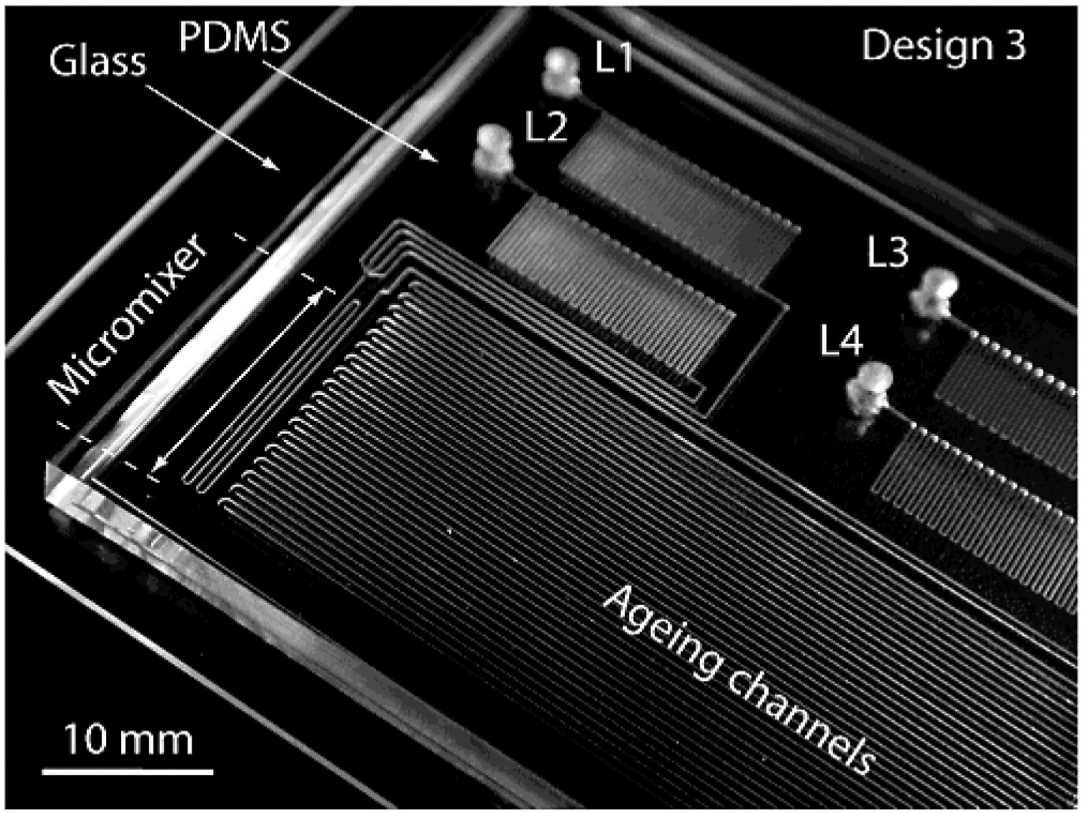

Figure 3.3: A PDMS and glass microreactor. Reprinted (adapted) with permission from Mason et.al ©2007 American Chemical Society

industries, which is otherwise limited by conventional batch reactors and their associated higher chemical inventory and safety concerns [39].

Though free enzyme catalysis is possible in the microreactor, one key component is the immobilization of enzymes, with a wide range of techniques having been reported. Physical immobilization of the enzymes on the surface of SiO_2 [68], PDMS [69] and fused silica [70, 71] has been successful. In all the cases, the enzymes were protected from getting denatured and could be used for multiple cycles without a significant loss in the enzymatic activity. A higher enzyme loading has also been achieved with fused silica as a higher surface area was available within the sol-gel modified surface although these techniques require several steps and are limited to a narrow range of enzymes. Enzyme entrapment [72] and encapsulation [73] (within a polymer matrix or silica) followed by immobilization on the surface of micro-reactor channels is another well reported technique. These microreactors were compatible with organic solvents and were used over multiple reaction cycles, although the preparation involved several time consuming steps and the enzymes were often denatured during immobilization. Adsorption [74] and cross-linking [75] of enzymes incorporated within the walls of a microreactor has shown to provide solvent compatibility and longer enzyme activity. However, enzyme leaching and reduction in the enzyme functional groups is a common problem with this method.

The applications of enzymatic microreactors can be classified based on (i) catalytic biotransformation of substrates and (ii) kinetic screening of substrate molecules. An example of free enzyme reaction is electrophoretically mediated microanalysis, which is carried out by making use of different zones of mixing within the microchannel based on the mobility of the enzyme and the substrate molecule [76, 77]. Exciting opportunities include the tandem synthesis of pharmaceuticals using two microreactors in series. This exhibited higher conversions at lower reaction times in comparison to batch reactors in series [78]. Another interesting application is for complex reactions like chemo-enzymatic synthesis, which has converted simple substrates to polymers [79]. A recent application is the production of biopolymers [8], which resulted in a higher molecular weight bio-polymer in comparison to the batch reactor. The effect of inefficient chain propagation as a result of diffusion limitation in the batch reactor was eliminated in the microreactor.

Microreactors are a promising class of reactors for a transition from traditional reactor modules to continuous flow reactors for industrial manufacturing. Some of the key challenges [80] that need to be addressed are (i) clogging of channels with particles and slurries to ensure stable operating conditions, (ii) lack of computational tools and adequate information of reaction mechanisms for achieving a better control over process optimization in the reactor and (iii) difficulty in implementing "plug and play" reactor configuration as the reactor design is not universal. Currently, the production scale-up of low cost chemicals in a microreactor is not economically viable, but the operation cost is justified for high value chemicals as the enzymes are recycled, and so they can be considered as a potential opportunity by industry. The future scope for the reactor lies in engineering continuous and sustainable processes by improving existing protocols for multistep reactions, accurate reaction control to further enhance the selectivity of the reactor, reducing the reactor design time and cost by using active screening parameters (temperature, pH, substrate concentration, etc.) and application to multiphase reactions that are usually limited by low conversion and rapid enzyme deactivation.

3.3.2.3 Immobilized Monolithic Enzyme Reactors (IMERs)

Monoliths are structural supports, comprising of well-defined capillary channels. The enzyme is immobilized by either wash or dip coating on the inner walls of the monolith, aiming to retain the porosity of the support and achieving a high enzyme activity (Figure 3.4). Traditionally, monolith catalytic reactors have been used for emission control from automobiles. However, their application is becoming more diverse and they are now widely applied in the chemical and biochemical industries owing to the range of advantages of these reactors [12, 81]

- One of the main features is the reduction in pressure drop by a couple of orders of magnitude when compared to the PBRs.

- A higher surface area, 1.5 to 4 times higher than reactors with catalyst pellets, which results in enhanced reaction rate and conversion.
- The catalyst efficiency is improved due to the shorter diffusion channels and a reduction in the mass transfer resistance. Reaction selectivity is also improved as it is easier to control the reactions.
- They also facilitate process miniaturization as they can be portable and help in mobile applications like ethanol reforming and cleaning of aircraft cabins. Development of small monolithic reactors also find application in fast screening of drugs in pharmaceutical industries.

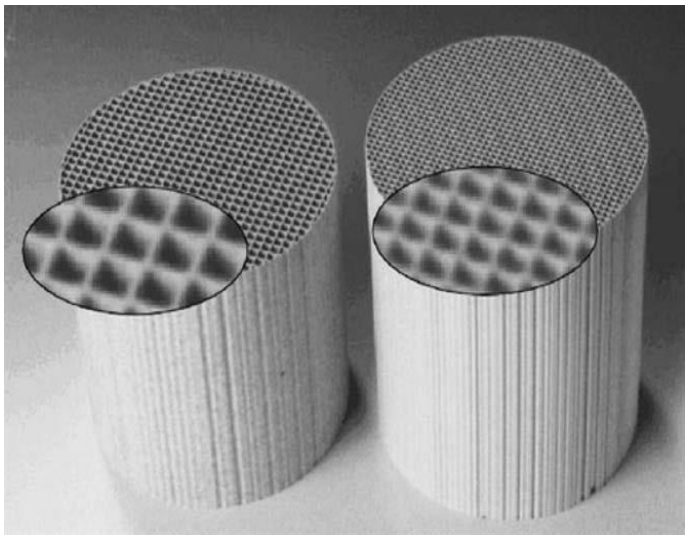

Figure 3.4: Monolithic supports for enzyme immobilization. Reprinted from Lathouder et.al ©2006, with permission from Elsevier

Monolithic supports can be either be organic polymers or inorganic monoliths. Organic monoliths are more popular as they are stable over a wide range of operating conditions, and have abundant functional groups, which facilitate efficient and simple enzyme immobilization protocol, and are also biocompatible. The use of epoxide groups (with or without modification) in a monolith for enzyme immobilization has been well reported in literature [82–84]. The enzymes retained their activity for a longer period, accompanied by a lower pressure drop, compared to the PBR. Monolithic Convective Interaction Media (CIM discs) are commercially available monoliths (BIA separation) and have also been used for enzyme immobilization [85, 86]. Silica-based monoliths are the most popular inorganic support for enzyme immobilization as they offer a macroporous channel and mesoporous skeleton, which enable higher enzyme loading, fast mass transfer and lower pressure drop [87]. Composites [88], modified silica [89, 90] and sol-gel [91] networks are other monoliths, which have been used for enzyme immobilization. In all cases, enzyme activity

was retained over a longer time period, due to the inherent structural stability provided by the monolith.

Application of IMER for production of biodiesel has been reported in literature using both inorganic and organic monoliths. Silica [92] and acrylic [85] based IMER have been used for enzymatic transesterification of oil, resulting in high product yield and selectivity compared to the free enzyme catalysed reaction. IMERs have also been used in chemical synthesis. Terpene was synthesized using lipase immobilized on a styrene based monolith, and it out-performed the PBR [44]. Another successful application was the production of lactose-free milk using β-galactosidase immobilized on lab-made agarose column [93]. Use of IMERs in pharmaceutics has also shown to provide the advantage of fast screening of drug molecules and drug development, and since the majority of the targets are enzymatic in nature, the enzyme immobilized monoliths can alleviate the need for complex in vitro characterization tests. CIM based IMERs immobilized with acetylcholinesterase have been extensively used in the therapeutic research for Alzheimer's disease [94, 95]. The advantage with the IMER was that it provided the necessary structural stability for the enzyme, and over 2000 individual tests could be carried out without a loss in the enzymatic activity. Finally, a widely reported application of IMERs is the digestion of proteins by enzymes. Compared to the conventional time consuming in-solution digestion, IMERs facilitate protein digestion within a few minutes, and also allow automation as they can be integrated within the system. Immobilized trypsin has for example been used for the digestion of cytochrome [84], BSA [96] and peptides [97]. Hybrid organo-inorganic monolith has been used for automated digestion of insulin and lysozyme [58].

Despite the number of advantages associated with IMERs, the technology is still novel and has not been employed for large scale industrial bioprocesses [81]. The monoliths are currently lab made, tailored for a specific enzyme and the scale-up of monolith production is a challenge in itself. The cost of manufacturing monolithic catalysts is higher than pelleted catalysts. However, the industries should consider the re-usability of monoliths, which can offset the high initial cost. Irreversible immobilization of enzymes on expensive monolithic supports hinders their re-usability once the enzyme deactivates and regeneration of such supports have to be considered.

3.3.2.4 Rotating Packed Bed Reactors

Rotating packed bed reactors (RPB) have been studied since the 1970s and facilitate intensified heat and mass transfer reactions generally applied for gas-liquid phase reactions. The application of the RPB reactor for enzymatic reactions was developed in the mid-90s and is still not well researched. They can be thought of as a combination of a rotor-stator and a static mixer in which the centrifugal force causes the fluids flowing through the packed bed immobilized with enzyme

to spread out as fine droplets or thin films, thus resulting in better mixing (Figure 3.5). Some of the characteristics of the RPB are as follows [98]:

- Mass transfer resistances are reduced due to the centrifugal force causing the formation of a thin film completely wetting the packed enzyme bed.
- The residence time of the RPB is in the order of 10 to 100 μs enabling them to be employed for fast reactions.
- Enzymes activity is retained over a longer period as they are protected from the hydrodynamic forces within the reactor.

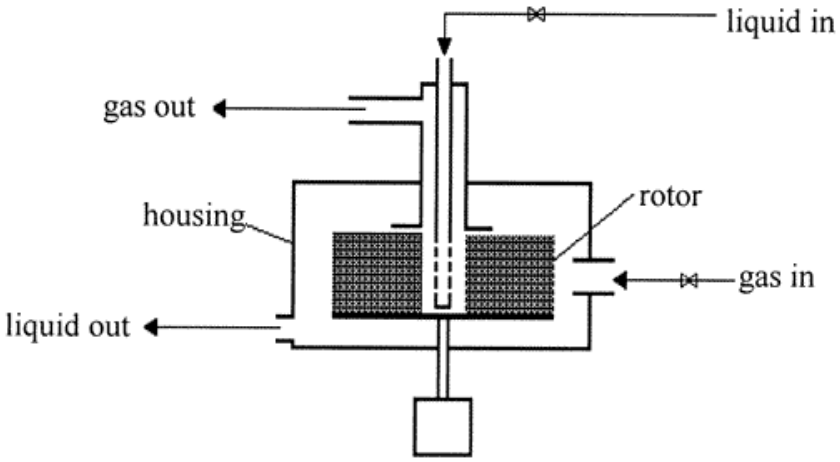

Figure 3.5: Schematic diagram of a rotating packed bed reactor. Reprinted (adapted) with permission from Liu et.al ©1996 American Chemical Society.

The RPBs have to date been used for nanofabrication [99], advanced oxidation [100, 101] and biodiesel production [102]. More recently, the RPBs have been extended for enzymatic biotransformation reactions[103–105] like glucose oxidation and oil hydrolysis. For example, the importance of rotational speed was demonstrated by a 20% increase in production rate with increasing speed for glucose oxidation [103]. The RPB has also been successfully employed for a three phase reaction system, which proved to be more efficient than a conventional fluidized bed reactor in terms of energy efficiency and reducing the attrition of the immobilized catalyst particles. Enzymatic conversion of rifamysin B to rifamycin S, an inflammatory drug, proceeded faster than in the RPB [104]. The efficiency of the degumming of rice bran oil using immobilized Lecitase [105] was increased with the impeller speed retaining the enzymatic activity.

The application of RPB to enzymatic reactions is still limited. Reactor scale-up requires an increase in the packed bed size, leading to reduced reactor efficiency due to incomplete wetting of the packing. The energy and maintenance costs of the rotating system are currently higher than conventional columns, which can be offset by a reduction in the size and the capital cost of the

reactor. The future prospects for the reactor are in terms of advances in design and process control for continuous reactions and extension into more complex enzyme catalysed reactions.

3.3.2.5 Spinning Mesh Disc Reactor (SMDR)

The Spinning Mesh Disc Reactor (SMDR) is an innovative reactor design developed by the author. Like the spinning disc reactor (SDR) technology, the SMDR also uses centrifugal force for an even spread of thin film on the surface of the disc, but additionally holds a cloth immobilized with the enzyme (Figure 3.6). The cloth is a critical component as it allows for improved mixing on top as well as within the cloth, which increases the contact between the enzyme and the reactants [27], and thus, creating a highly localized reaction zone.

The key advantages of the SMDR are [27, 66]:

- Reaction intensification occurs through a combination of increased mass transfer and interfacial surface area.
- The cloth protects the enzymes from hydrodynamic forces and the enzyme activity is retained over multiple cycles.
- The residence time is in the order of milliseconds, which facilitates fast reactions.
- Mild operating conditions allow for reactions in aqueous and organic solvent medium.

The SMDR has been used to study the hydrolysis of tributyrin using free and immobilized lipase on wool [27]. In both cases, the reaction conversion was higher than the BSTR. The reaction conversion also increased with the increase in spinning speed up to a certain threshold, beyond which enzyme leakage was observed, likely due to the surface shear on the cloth surface. The enzyme cloth was re-used for 15 cycles and 80% of the original activity was retained, demonstrating the robustness of the reactor. It has also been shown that any cloth support used for enzyme immobilization can be incorporated in the SMDR, paving way for different structural supports to be integrated with this technology. The improved mixing in the SMDR can be attributed to the number of tanks in series, which is two times lower than reactor without the cloth. Scale-up of the SMDR can be achieved by increasing the number of immobilized cloths and numbering up of the discs [22]. Similar to the scale-up of microreactors, scale-up of a SMDR in general does not require a re-design in terms of operating co-efficient like Prandtl and Reynold's numbers, but is solely governed by physical laws [65]. The reactor has recently been extended to chemical synthesis, producing nitroalcohol from aldehydes [106].

Due to the recent development of the SMDR, there has not been a technology transfer to the industry. The SMDR is as a niche and flexible technology, suitable for the production of pharmaceuticals and fine-chemicals. Future research should focus on extending the SMDR technology

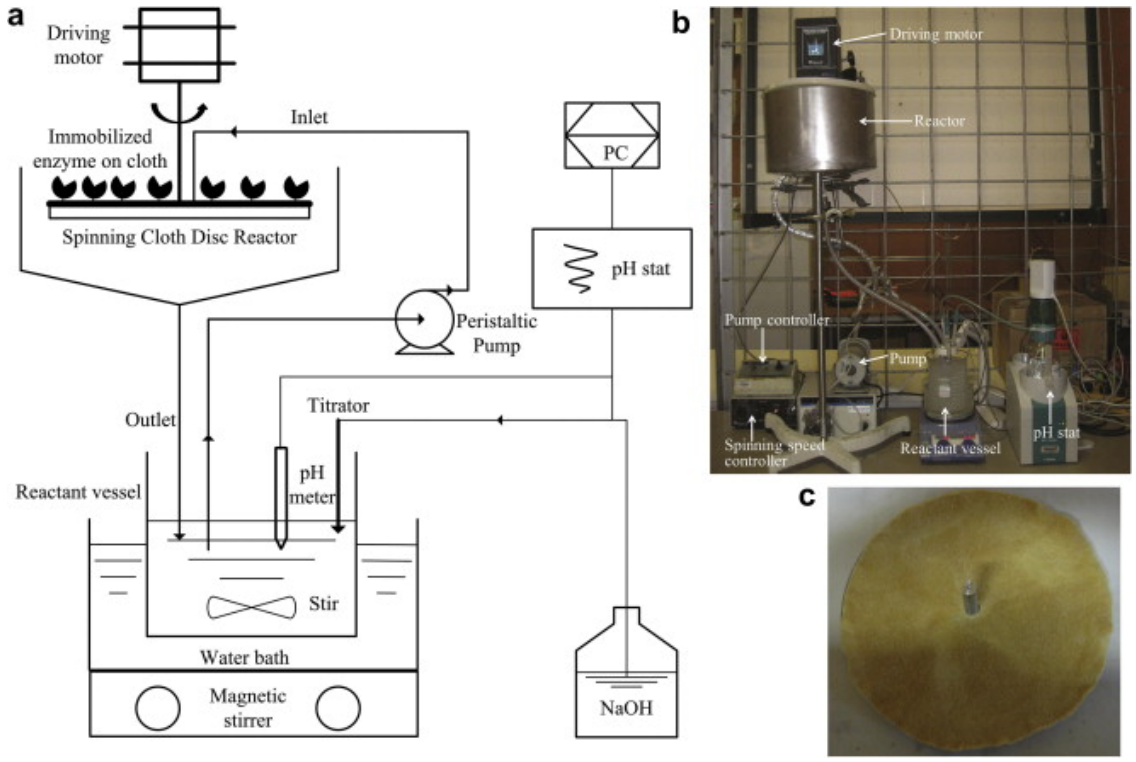

Figure 3.6: (a) Schematic diagram of the SMDR for tributyrin hydrolysis, (b) Photograph of the reactor set-up, (c) Enzyme immobilised on a woollen cloth support. Reprinted from Feng et.al ©2013, with permission from Elsevier

to organic synthesis, cascade and multifunctional reactions by integrating alternate forms of energy. Parallel operation of multi-disc design can also be one of the ways to achieve the production demand.

3.4 Conclusion

Process intensified enzyme reactors have been successful in surpassing the performance of conventional reactors for biotransformation on a lab scale, justifying the academic and industrial attention they have received in the last decade. Reactor engineering coupled with a fundamental understanding of the chemistry has resulted in improved efficiency of enzyme reactions developing a range of PI-ERs. While membrane and micro reactors have been tested for a range of applications, RPB and SMDR have the potential versatility to transform novel, bench scale chemistry into continuous processes. The future scope lies in integration of PI domains in the form of alternate energy source, multi-functionality and synergistic reactions. The next steps for industrial implementation of these technologies also include cost assessment, process control, design and development of the reactors for higher throughput. Another possible means of ensuring faster technology transfer to industries is by commercialization of PI-ER research as spin-out companies. To conclude, the

combination of process intensification and enzyme catalysis is an effective way of achieving sustainable processes on an industrial scale, and this can only be achieved if engineers and chemists work together at all the development stages, rather than as an afterthought.

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Chapter 4

Kinetic resolution in the Spinning Mesh Disc Reactor

Enzymes are increasingly becoming the choice of catalyst in the pharmaceutical industries compared to conventional metal or metal organic catalysts as they: (i) enable high reaction selectivity and (ii) minimise side reactions, hence reducing the amount of waste generated making it an environmentally benign process [1]. However, scale-up of enzymatic reactions in conventional reactors results in high mass transfer resistance and intensive catalyst recovery steps reducing the overall process efficiency [2]. Hence, there is a need for intensifying these reactions for improved productivity and better enzyme recoverability.

Among the many process intensified reactors (as seen in Chapter 3), the SMDR has shown potential for intensification of organic reactions. Hence, the next step is to extend the application of the SMDR for intensification of enzyme catalysed organic synthesis. Kinetic resolution of a racemic alcohol was chosen as the model reaction, as the reaction end product has application in the pharmaceutical industry and the reaction is well reported in literature for easy comparison.

The following paper presents a detailed study of kinetic resolution of 1-phenylethanol using immobilised lipase in the SMDR. Lipase was first immobilised on wool and the resulting lipase cloth was fully characterised to establish the presence of lipase. The reaction was tested in batch using both free and immobilised lipase using a range of different solvents and temperatures. The optimised reaction condition in batch was then incorporated for the reaction in the SMDR. Various reactor parameters were tested for reaction optimisation in the SMDR. Re-usability of the catalyst cloth was also tested for the reaction. Additional plots for this chapter can be found in Appendix B.

<table>
<tr><td colspan="10">This declaration concerns the article entitled:</td></tr>
<tr><td colspan="10">Kinetic resolution of 1-phenylethanol in the spinning mesh disc reactor: Investigating the reactor performance using immobilised lipase catalyst</td></tr>
<tr><td colspan="10">Publication status (tick one)</td></tr>
<tr><td>draft manuscript</td><td></td><td>Submitted</td><td></td><td>In review</td><td></td><td>Accepted</td><td></td><td>Published</td><td>✓</td></tr>
<tr><td>Publication details (reference)</td><td colspan="9">Shivaprasad, P., Jones, M. D., Patterson, D. A., & Emanuelsson, E. A. C. (2018). Kinetic resolution of 1-phenylethanol in the spinning mesh disc reactor: Investigating the reactor performance using immobilised lipase catalyst. Chemical Engineering and Processing - Process Intensification, 132, 56–64.</td></tr>
<tr><td>Candidate's contribution to the paper (detailed, and also given as a percentage).</td><td colspan="9">Formulation of ideas (60%): Emma Emanuelsson and Darrell Patterson developed the reactor. Matthew Jones and I suggested the reaction scheme as it was a straightforward mechanism and could be coupled with the Henry reaction to later investigate the cascade reaction. Matthew Jones contributed to formulating the batch studies as the reaction was not straightforward with the immobilised catalyst. Emma Emanuelsson and I formulated ideas for reaction in the SMDR.

Design of methodology (70%): I planned the characterisation studies based on that carried out in Chapter 2. Matthew Jones suggested the outline for batch studies and I contributed to testing a wider range of parameters. I mainly designed the methodology for the reactions in SMDR and Emma Emanuelsson contributed to additional experiment plan to further improve the optimisation study.

Experimental work (95%): I performed all the experiments in batch and the SMDR. SEM, EDX and XPS analysis of the samples were carried out by the technical staff. I also carried out most of the data interpretation after subsequent discussions with my co-authors.

Presentation of data in journal format (80%): I mainly prepared the manuscript for the journal including graphics in journal format, data interpretation and incorporating reviewer comments. The co-authors contributed to revising the individual sections at the draft stage.</td></tr>
<tr><td>Statement from Candidate</td><td colspan="9">This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.</td></tr>
<tr><td>Signed</td><td colspan="5">Parimala.S</td><td>Date</td><td colspan="3">10/01/2019</td></tr>
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Abstract

The spinning mesh disc reactor (SMDR) is an innovative catalytic rotating reactor to aid process intensification. In this study, the application of the SMDR has been demonstrated for the enzymatic kinetic resolution of racemic 1-phenyethanol using amano lipase immobilised on wool as a catalyst. Physical characterisation of wool was carried out to confirm the presence of lipase. The reaction was tested for a range of solvents and temperatures for both free and immobilized lipase and the optimised reaction conditions were employed in the SMDR for different flowrates and spinning speeds. The SMDR showed better reaction efficiency compared to the batch reactor: the feed throughput was scaled-up from 10 ml to 250 ml and the productivity increased from 7.05 g l^{-1} h^{-1} in batch to 10.92 g l^{-1} h^{-1} in the SMDR. An increase in catalyst loading was achieved by adding more lipase cloths and the reaction rate increased from one cloth (0.16 mmol min^{-1}) to three cloths (0.28 mmol min^{-1}). These results show the first demonstration of novel reactor design for scale-up of enzymatic kinetic resolution using an inexpensive lipase. The SMDR thus shows potential for scale-up and continuous processing for versatile applications in the fine chemicals and pharmaceutical industry.

4.1 Introduction

There is a demand for enantiopure compounds for bulk manufacture of pharmaceuticals and agrochemicals [1]. Biotransformation of racemates into enantiomers using enzymes is an attractive option owing to the high regio, enantioselectivity and a safer alternative to chemical synthesis [3, 4]. The difference in the reaction rates of the enantiomers with the enzyme (chiral catalyst) results in the resolution of the racemates. One such important reaction is the resolution of 1-phenylethanol via acylation using a suitable acyl donor and catalysed by lipase, as the chiral derivatives of phenylethanol are often used as starting materials in the pharmaceutical and natural products industries [5–10]. Non-amano lipases have been studied extensively for this reaction as they show high selectivity and product yield [7, 9–12]. However, they are very expensive and not economical for process scale-up. An alternative is to use inexpensive amano lipases for kinetic resolution, although in comparison they have shown maximum conversions of approximately 30% and 97% enantioselectivity [6]. Other challenges with this reaction are prolonged reaction times, low recoverability of expensive enzymes, solvent compatibility and process scale-up [12, 13].

The spinning disc reactor (SDR) is a promising process intensification technology in which the centrifugal force associated with the spinning disc causes the liquid feed impinged on the centre to spread out into a thin film with high surface shear [14, 15]. This is responsible for rapid mixing, accompanied by short residence times for reactions and improving the overall heat and mass transfer [16]. The application of a SDR has been well reported in literature for a wide range of chemical processes, such as precipitation, polymerisation, photocatalytic reactions, production of pharmaceuticals and synthesis of nanoparticles [16–21]. The spinning mesh disc reactor (SMDR) is a novel reactor built on a similar concept to the SDR, but additionally houses a cloth with an immobilised catalyst attached on the disc surface allowing the centrifugal force of the spinning disc to create a thin film over and within the cloth. This improves mixing and mass transfer within the film, accelerating the reaction rate, as well as protecting the catalyst from the shear forces associated with the spinning disc [22]. The SMDR is characterised by two key design parameters, the spinning speed and the feed flow rate. Optimisation of these parameters are achieved based on the effect of the shear forces on the catalyst stability and the optimal flowrate is determined based on the catalyst activity and concentration.

Textile based supports have diverse structural and mechanical properties and are cheap [23]. Wool has shown effective support properties for both biological and non-biological catalysts as it is rich in surface functional groups, allowing the catalysts to be firmly bound onto the surface, offers a high surface area and is a sustainable alternative to synthetic supports [24–26]. Wool has been demonstrated as a catalyst support in the SMDR so far for two reaction systems, enzymatic hydrolysis of tributyrin (water-enzyme) [22, 27] and copper catalysed Henry reaction (organic

solvent-metal catalyst) [25]. Intensification of enzymatic hydrolysis of tributyrin using immobilised lipase was achieved successfully in the SMDR and both the rate and the final conversion increased compared to a traditional batch reactor. Liquid flow characterisation studies indicated that the flow patterns in the SMDR was analogous to a well-mixed reactor in contrast to the plug flow behaviour of the SDR [27]. The catalyst loading was easily increased by increasing the number of cloths with the immobilised lipase on the disc. We also showed that the SMDR is suitable for traditional organic synthesis, as demonstrated by the Henry reaction catalysed by copper triflate immobilised on wool, achieving an increase in both final conversion and rate [25]. An additional advantage with the SMDR is the use of immobilised catalysts which enables easy catalyst separation, recovery and reuse. One of the aims of this research is therefore, for the first time, to achieve process intensification of enzyme catalysed synthesis in organic solvents. This will also further demonstrate the versatility of the SMDR. Also, to the best of the author's knowledge, enzyme catalysed organic synthesis in other process intensified reactor, like microreactors have been investigated on a small scale ($\sim$ 10 ml) and the present study demonstrates the application of the reaction on a larger scale [28].

The aim of the present work is thus to: (i) demonstrate the potential of the SMDR for kinetic resolution of 1-phenylethanol using amano lipase immobilised on wool as a catalyst, (ii) optimise the reaction conditions in the SMDR based on flow rate and spinning speed. Physical characterisation will be carried out to verify immobilisation of lipase on wool. Reactions in batch will be carried out using a range of different solvents and temperatures to determine the optimum condition for use in the SMDR for both free and immobilised lipase.

4.2 Materials and Methods

4.2.1 *Materials*

Unbleached wool (1.5 mm thickness, cream colour) was obtained from Urbanara (Berlin, Germany). All chemicals, lipase from *Pseudomonas fluorescens* (PF) and solvents were purchased from Sigma Aldrich and used as received unless specified. All solutions were prepared using deionised water (Elga).

4.2.2 *Lipase immobilisation on wool*

A detailed immobilisation procedure has been published elsewhere [29]. In summary, woollen cloth was cut into circular pieces (12 cm diameter). The cloth was pre-treated using a solution containing hydrogen peroxide and sodium silicate in a pH 9 carbonate buffer for 70 minutes. Surface modification of the pre-treated cloths was carried out by dipping the cloths in 2% PEI

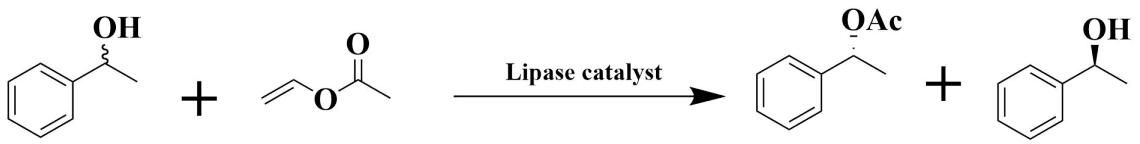


Figure 4.1: Kinetic resolution of 1-phenylethanol with vinyl acetate as the acyl donor, catalysed by lipase.

(polyethylene imine) solution at pH 8 for 2 hours at room temperature. The cloths were thereafter rinsed with deionised water and soaked in 2 gL^{-1} lipase solution in pH 6 phosphate buffer for 24 hours. The lipase immobilised cloths were then crosslinked using 0.5% glutaraldehyde in pH 6 phosphate buffer for 10 minutes and rinsed with deionised water to remove excess enzyme and crosslinking solution. The lipase activity was measured using the tributyrin emulsion method [22] and was found to be 248 Ug^{-1} of cloth, where one enzyme unit (U) is defined as the amount of lipase that catalyses the release of 1 μmol of butyric acid per minute.

4.2.3 *Material characterisation of wool*

Lipase wool was characterised by Fourier transform infrared spectroscopy (FTIR) using a Perkin-Elmer-100 FTIR spectrometer. Plain and lipase wool samples were scanned between the wavelengths 4000 to 500 cm^{-1} without any further sample preparation. The background scan was carried out without any sample before the samples of interest were scanned. The surface morphology and elemental composition studies of plain and lipase wool were done using SEM (JEOL SEM6480LV) and EDX (Oxford INCA X-Act SDD) respectively. Individual fibres of plain wool and lipase wool were stuck on a double sided carbon tape and coated with gold to improve the imaging quality for SEM. Cross section of wool fibres were used for EDX analysis. Individual wool fibres were stuck on a double sided tape and embedded in Epotin Z resin, mixed with the corresponding hardener. The samples were cured for 12 hours in vacuum and polished with a diamond polisher (Buehler Ecomet 250 Pro) and finally washed with soap and ethanol. The resin surface was coated with chromium (5 nm) before the analysis. The surface composition of plain and lipase wool was analysed by X-ray photoelectron spectroscopy (XPS) using a Thermo k-alpha+ system at the XPS facility at Cardiff University. Fibres of plain wool and lipase wool were stuck on a double sided tape of 1 cm^2 and used for analysis.

4.2.4 *Kinetic resolution of 1-phenylethanol in batch*

In a typical batch reaction, 1-phenylethanol (0.5 mmol), vinyl acetate (2.5 mmol) and the desired amount of catalyst were added to a suitable solvent (3 ml) and stirred (Fig 4.1). The reactions were carried out in a reaction carousel for 24 hours. Control experiments were carried out without

the catalyst under the same conditions and no reaction occurred. Samples were drawn periodically and the reaction conversion was monitored using gas chromatography (Varian CP-3800, CP-Sil-8 CB column). Enantiomeric excess was measured using HPLC.

4.2.5 *Kinetic resolution of 1-phenylethanol in the SMDR*

As shown in Fig 4.2, the SMDR consists of an overhead liquid feeding system, a disc connected to an overhead stirrer and a funnel like vessel to channel the spin-off feed from the edge of the disc to the reactant storage vessel. The cloth was placed on a glass disc and connected to a rotor (Heidolph RZR 2021), operating at variable speeds. Fresh lipase cloths were used for each study. Further details about the reactor set-up can be found in our previous publication [25].
1-phenylethanol (0.5 mmol), vinyl acetate (2.5 mmol) were dissolved in 250 ml of solvent and the reaction was caried out at 25°C for 5 hours. Samples were drawn every 5 minutes for the first 30 minutes and once every hour thereafter. The samples were analysed using GC as above and the

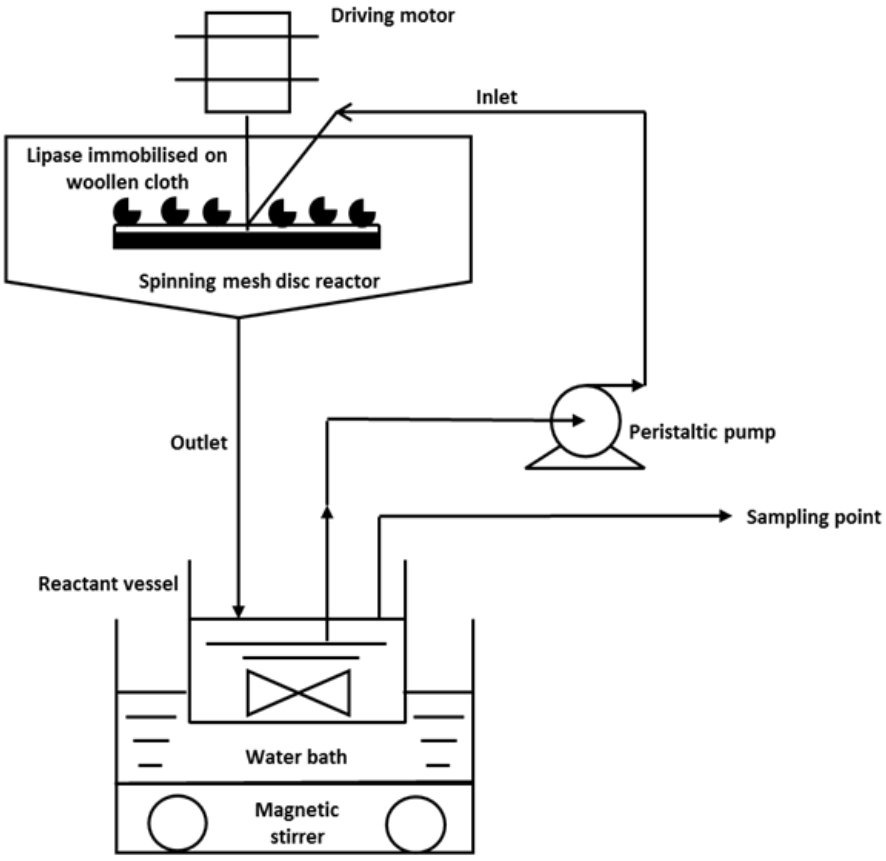


Figure 4.2: Schematic representation of the SMDR with the lipase cloth placed on the disc

sample at the end of the reaction was also analysed using NMR (300 MHz Bruker Spectrometer). Analysis of ^{1}H NMR integral of 1-phenylethanol at 4.72 ppm to the integral of 1-phenylethyl acetate at 5.79 ppm was used to calculate the conversion.
The reactor performance was characterised based on the average shear on the surface of the disc

($\bar{S}$) given by the following equation:

$$\bar{S} = \frac{1}{R}\int_0^R Sdr = \frac{3}{4}\left(\frac{3QR\omega^4}{2\pi\nu^2}\right)^{1/3} \tag{4.1}$$

Where, $\bar{S}$ = shear stress (s^{-1}); Q = Volumetric flow rate (m^3 s^{-1}); R = Radial distance (m); ω = Angular velocity (rad s^{-1}); ν = Kinematic viscosity (m^2 s^{-1})

An increase in the spinning speed results in a proportional increase of the average surface shear and thus an increase in the conversion is expected. However, research has shown that enzyme leaching from the woollen cloth occurs over a certain value of average surface shear known as the critical surface shear, which is 9500 s^{-1} [30].

4.2.6 *High performance liquid chromatography (HPLC)*

The enantiomeric excess (ee) was determined by HPLC (Agilent Technologies 1260 Infinity-II), Chiracell OD-H column with Hexane/IPA (90/10) at 1 ml min^{-1} and 254 nm wavelength using a UV detector. The 'ee' of the reaction was calculated using the following equation:

$$ee = \frac{(S-R)}{(S+R)} \tag{4.2}$$

4.2.7 *Kinetic studies*

The ping-pong bi-bi kinetic model has been used successfully to describe the kinetics of the resolution reaction [31]. The enzyme first forms a complex with the acyl donor (acyl-enzyme), conforming to the ordered bi-bi mechanism. The complex then undergoes an isomerisation reaction to form the reaction intermediate, followed by the release of the alcohol product. The second substrate, 1-phenylethanol binds to the acyl-enzyme complex, undergoes an isomerisation reaction resulting in the formation of an ester-enzyme complex, followed by the release of the acetate product and the free enzyme. Furthermore, the substrates and the products are considered as competitive inhibitors. The rate expression is given by:

$$v = \frac{\frac{v_{max}}{K_m}S}{1 + \frac{S}{K_m} + \frac{(S_\circ - S)}{K_i}} \tag{4.3}$$

where, v is the reaction rate, v_{max} is the maximum rate of the reaction, K_m and K_i are the kinetic constants, S is the final concentration of the substrate and $S_\circ$ is the initial substrate concentration. The initial rate was calculated from the concentration-time profile for the reaction carried out at different temperatures and compared with the theoretical rate values obtained from the model.

4.3 Results and Discussion

4.3.1 *Characterisation of lipase immobilised wool*

FTIR, SEM, EDX and XPS was used to show that lipase was successfully immobilized on wool. The functional groups present in wool were determined by analysis by FTIR spectroscopy (Appendix B). Fig 4.3 shows the SEM images of plain and lipase immobilized wool. As seen in

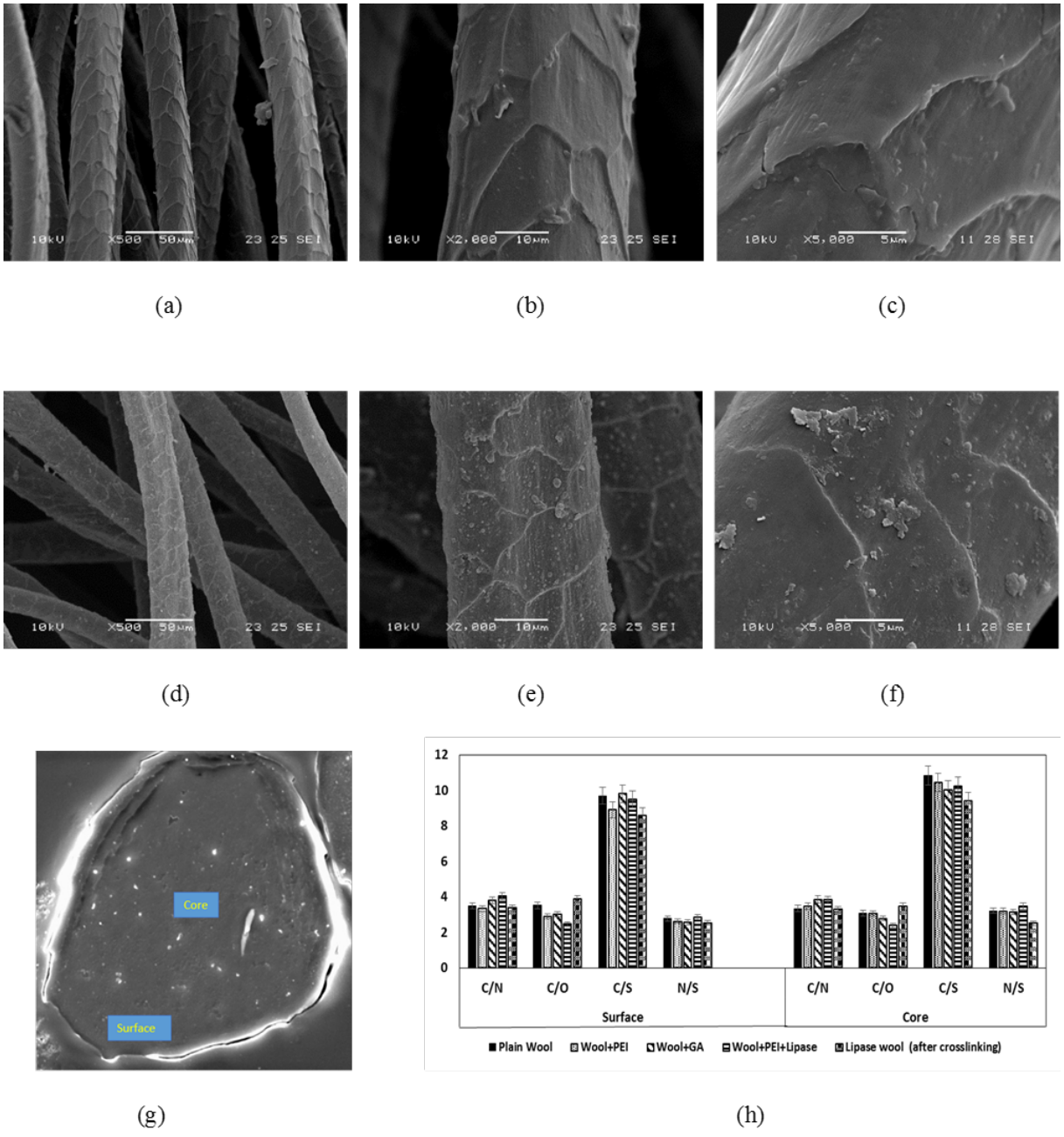


Figure 4.3: SEM images of (a-c) plain wool, (d-f) lipase wool and (g) SEM image of wool fibre cross section (h) Elemental ratios from the EDX analysis during different stages of lipase immobilisation

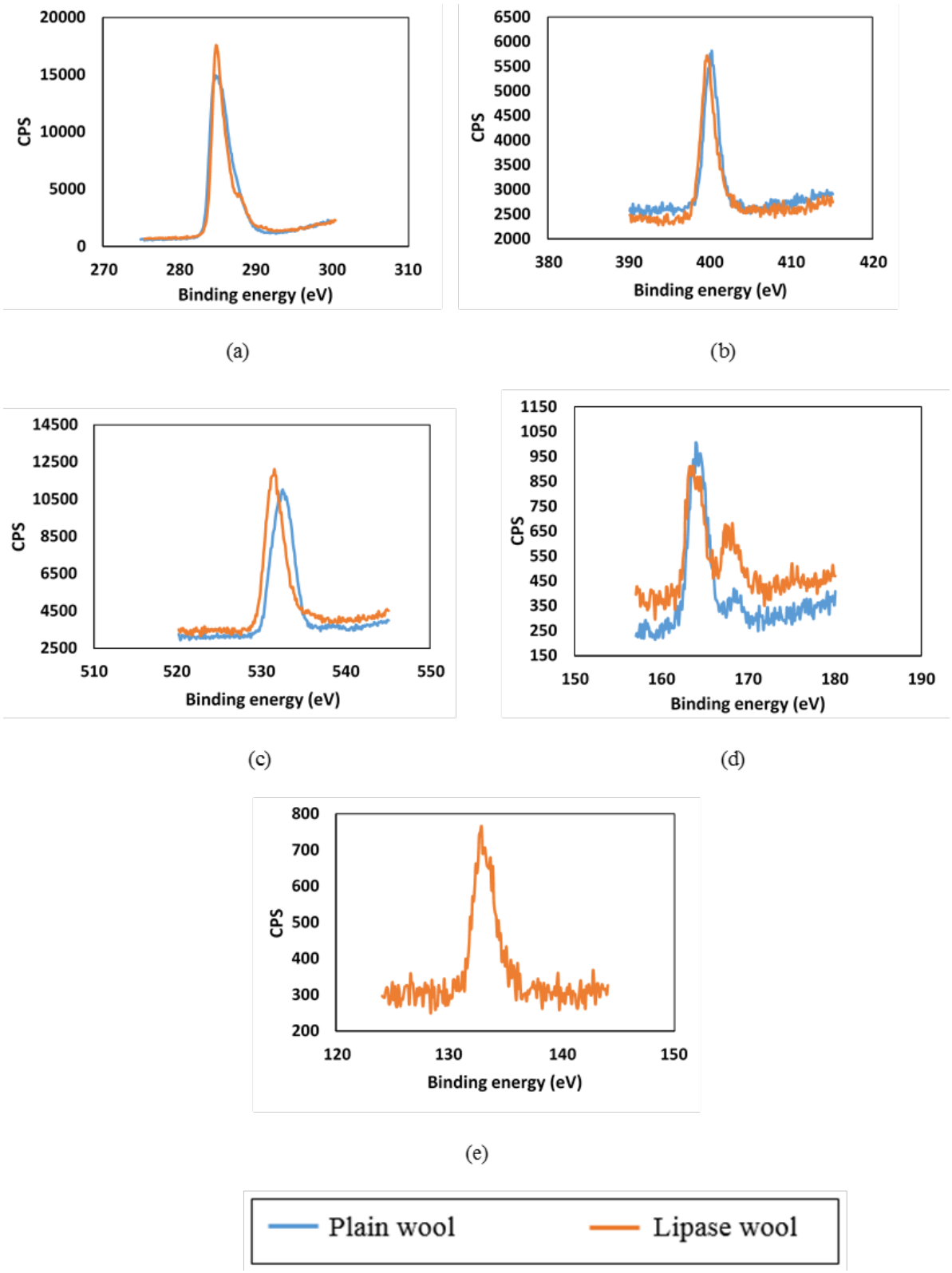


Figure 4.4: Elemental scans of plain wool and lipase wool measured by XPS: (a) C1s, (b) N1s, (c) O1s, (d) S2p and (e) P2p

Fig 4.3 (a-c), the morphology of plain wool is characterised by a uniform arrangement of cuticle cells on the surface of the wool fibres (diameter 2.5 μm). Changes in morphology of wool fibres

were observed at almost every stage of immobilisation (Appendix B). Fig 4.3(d-f) shows loss of surface smoothness after lipase immobilisation which may be due to a coating of lipase on the wool surface. Fig 4.3(g-h) shows the elemental ratios from the cross section EDX analysis from different stages of lipase immobilisation at the surface and fibre core. As lipase immobilisation occurs mainly on the surface of the wool, changes in the composition near the surface is more significant compared to the fibre core. Further, wool and lipase are mainly made of amino acids (containing carbon, nitrogen, oxygen and sulphur) and the reagents used for immobilisation have similar elemental composition. Thus, it would be expected to see a continuous increase in the carbon ratio throughout the course of immobilisation. Although it can be seen that this trend is not consistent throughout indicating possible interaction of the reagents with the surface functional groups containing sulphur and nitrogen resulting in decrease in carbon ratios at different stages of immobilisation.

The survey spectrum for plain wool obtained from XPS analysis indicated the presence of carbon (78%), oxygen (12%), nitrogen (7%) and sulphur (2%) (Appendix B). In difference, the survey spectrum of lipase wool showed the presence of phosphorous (2%), which further indicates successful lipase immobilisation on wool as only lipase of all the immobilization chemicals contain phosphorous. The elemental scans as seen in Fig 4.4, show a slight shift in the binding energy for O1s and S2p for lipase wool indicating a possible interaction of the immobilisation media and the surface functional groups present in wool.

4.3.2 *Effect of different solvents on catalyst performance and enantioselectivity in batch*

The choice of organic solvent for enzymatic resolution plays an important role as it affects the activity and the stability of the enzyme but also the substrate characteristics. Further, it has been reported that the enantioselectivity of the enzyme is significantly affected by the reaction solvent as there can be changes in the structural conformation of the enzyme and the nature of the enzyme-substrate intermediate compound [32].

A range of solvents, both polar and non-polar, were investigated and their effect of conversion and enantioselectivity are presented in Table 4.1. It shows that in a non-polar solvent like toluene, free lipase exhibited good reaction conversion and enantioselectivity but only little conversion was observed with the immobilised lipase. Aromatic solvents like toluene show poor wettability of wool resulting in a reduced contact between the catalysts present on and within the woollen cloth. Hence, a lower reaction conversion was observed with immobilised lipase [33]. Table 4.1 further shows that there is no activity using polar organic solvents like ethanol and DMSO. Research has previously shown that they offer least diffusion resistance through wool fibres but have

Table 4.1: Effect of solvent on reaction conversion using free and immobilised lipase in batch reactions after 24 hours

Solvent	**% Conversion with free lipase**[a])	**%ee$_s^b$**	**% Conversion with immobilised lipase**[a])	**%ee$_s^b$**
Toluene	49	91	3	<1
Ethanol	<1	-	0	-
Ethanol+Toluene	<1	-	0	-
DMSO	-	-	-	-
DCM	28	93	10	20
Ethyl acetate	48	92	30	23

[a]Conversion was determined by ^{1}H NMR analysis. The maximum conversion possible is 50% as the starting material was racemic.

[b]The enantiomeric excess was determined by chiral HPLC.

been reported to cause an activity loss of enzymes by denaturing them, resulting in no conversion and enantioselectivity for the reaction [33, 34]. As toluene demonstrated the highest conversion for free lipase and ethanol is known to have better penetration through the wool fibres, a combination of ethanol and toluene in equal volume proportions was also examined as a reaction solvent. However, as per Table 4.1, there was no change in the conversion indicating that the enzyme was denatured in the presence of ethanol. Moderate conversion and enantioselectivity was observed using DCM for both free and immobilised lipase. For ethyl acetate, similar conversions to toluene was observed using free lipase, but higher conversion for the immobilized lipase was achieved. Though there is only a marginal difference in the enantioselectivity for ethyl acetate and DCM, the former was chosen as the solvent for the reaction as it has a higher boiling point than DCM, minimising solvent losses and is also a more environment friendly solvent. Thus, all further experiments were carried out using ethyl acetate.

The results from the present study for both free and immobilised lipase are in line with that reported by de Souza et.al [6], using free lipase from PF for the same reaction system. As mentioned in the earlier sections, kinetic resolution of racemic alcohols have been mainly carried out using lipases from Candida species as employed in Novozyme 435 and other non-amano lipases due to their higher enantioselectivity. Amano lipase from PF was chosen for the present study as it was readily available, inexpensive and has been successfully immobilised on wool in the past [30]. In the present study, it is also a trade-off between solvent compatibility with lipase immobilised on wool and obtaining measurable conversions for the reaction to demonstrate the potential of the SMDR for an enzyme catalysed reaction, as seen in later sections. Also, other lipases have not

been screened for this reaction as it is out of scope for the present study as the main focus is to demonstrate reaction scale-up using an inexpensive lipase.

4.3.3 *Catalyst efficiency at different temperatures in batch*

To determine the best operating temperature for the catalytic activity of the lipase, the reaction in ethyl acetate was monitored between 25°C to 45°C. Maximum reaction conversion was achieved at room temperature for both free and immobilised lipase, and the conversion decreased with an increase in temperature (Fig 4.5). Our previous reports on hydrolysis of tributyrin using immo-

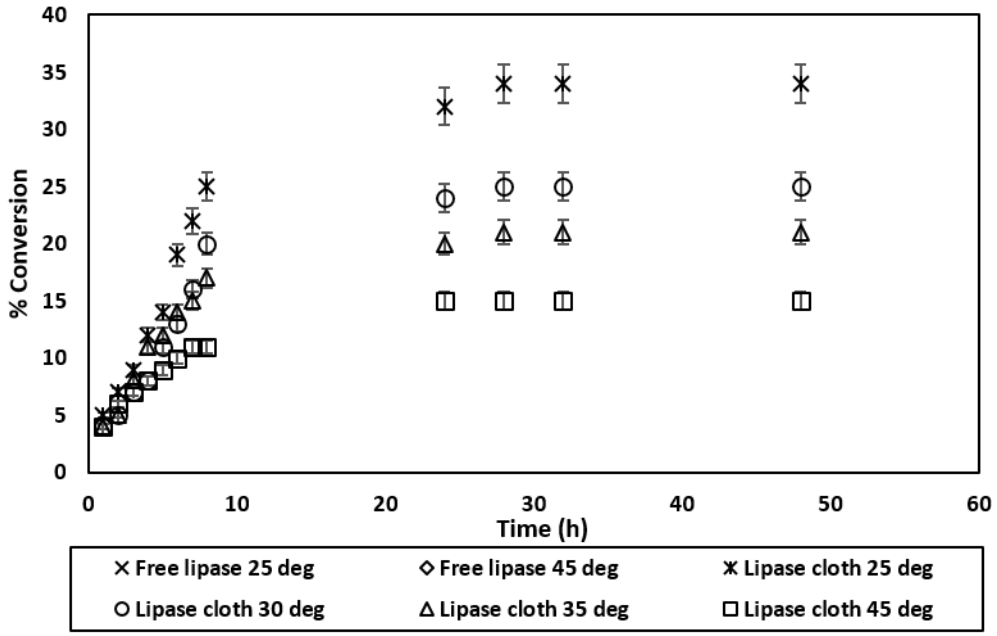


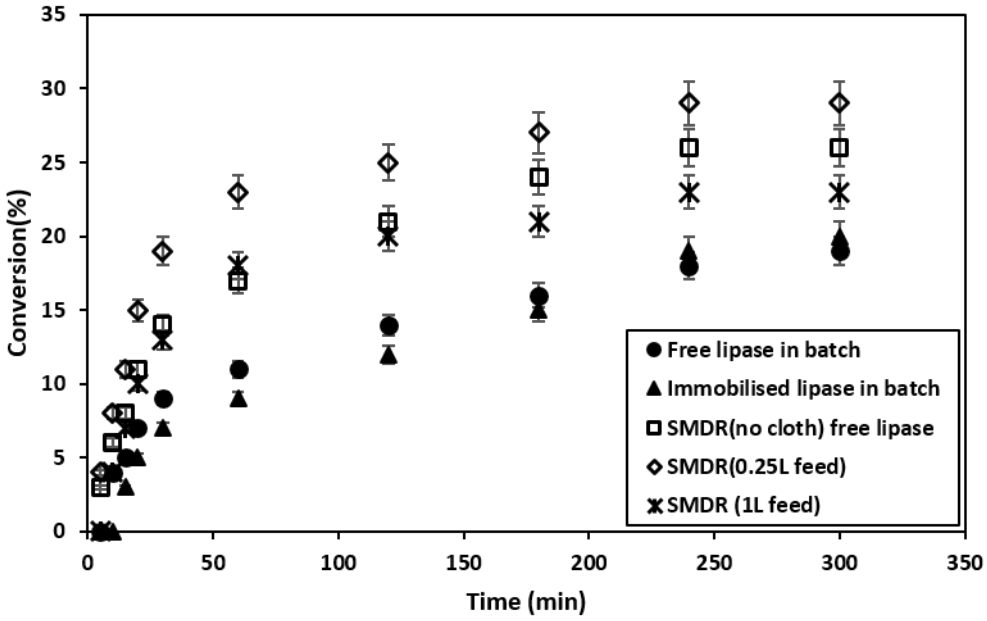
Figure 4.5: Effect of temperature on reaction conversion using free and immobilised lipase in batch

bilised lipase have shown an increase in the activity of the enzyme up to 55°C demonstrating the superior heat resistance of the lipase [22]. However, it was a non-enantioselective reaction and carried out in an aqueous medium. Traditionally, amano lipases (like pseudomonas fluorescens used in the present study) have shown maximum activity at room temperature for enantiomeric reactions as they are sensitive to the thermal effects of conventional heating process in an organic medium [7, 31, 35, 36]. They are also susceptible to temperature induced racemisation at higher temperatures resulting in reduced conversion and enantioselectivity [34]. It is natural to expect immobilised lipase to yield better conversions at higher temperatures as they are known to be more stable to thermal effects. In the present case however, in addition to temperature effect, reduction in conversion using lipase immobilised on wool may also be due to the alteration of the conformation of the activity centre of the enzyme during the immobilisation process [35].

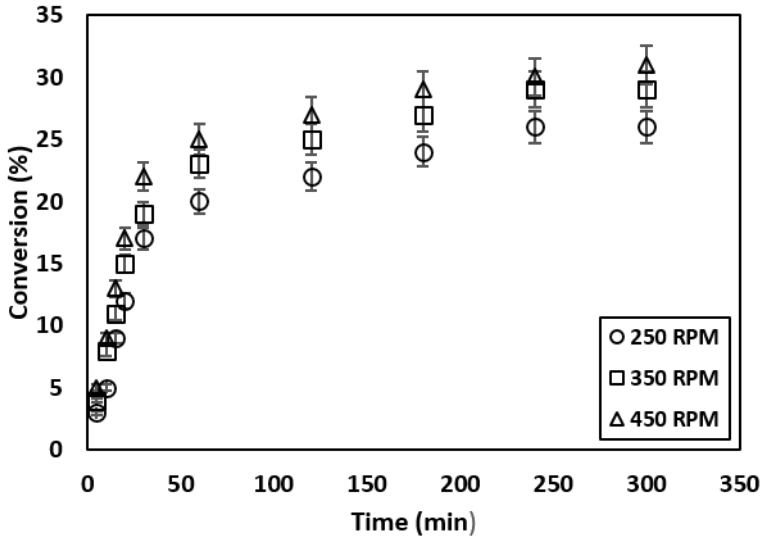
4.3.4 *Kinetic resolution in the SMDR using free and immobilized enzymes*

The SMDR was used for reaction scale-up and the reaction conversion was compared with that obtained in batch after 5h for 250 ml. Fig 4.6(a) shows that the reaction conversion increased for both free and immobilised lipase in the SMDR compared to the reaction in batch. Previous research [30] has shown that, in addition to overcoming mass transfer resistances, the cloth and disc geometry creates a higher interfacial surface area on the disc for the enzyme-substrate throughout the reaction and discrete residence time of the substrate on the spinning disc result in the enhanced reaction conversion using immobilised lipase in the SMDR. Hence, the lipase immobilised cloth was used for further reaction optimisation. The volumetric feed scale-up of the SMDR was tested by running a 1 L feed of the same concentration through the SMDR using similar disc size and reaction conditions. Although the conversion decreased by 20% compared to the 0.25 L feed, it was still higher than that achieved in batch using free lipase. Changing reaction volumes in the batch reactor has an effect on mixing and turbulence, in turn affecting the reaction rate. In the SMDR, the reaction volume only increases the volume of the substrate as the reaction does not occur in the bulk of the reactant vessel. The productivity in the SMDR using lipase cloth (10.92 g l^{-1} h^{-1}) was 35% higher than that obtained in batch (7.05 g l^{-1} h^{-1}). This is also higher than the productivity of 2.2 g l^{-1} h^{-1} achieved by Hartmeier et.al [37] using immobilised lipase in a fixed bed reactor.

Fig 4.6(b) shows that with the lipase immobilised cloth in the SMDR, reaction conversion increased with increase in the spinning speed of the disc, and a maximum conversion of 31% was achieved at 450 RPM in 5 hours, which is comparable with that in batch at the end of 24 hours. The centrifugal force associated with the spinning disc allows for an even spread of liquid film on and within the woollen cloth immobilised with lipase, resulting in better mixing within the film accompanied by reduced resistance to mass transfer and increase in conversion, in agreement with our previous results for enzyme hydrolysis [30]. It should be noted that although mass transfer resistance was successfully overcome in the SMDR, the lower overall conversion for the reaction is due to: (i) the solvent compatibility with the immobilised lipase woollen cloth, (ii) probable loss of chiral active centres of the lipase as a result of its immobilisation on wool. As can be seen from Table 4.2, the reaction conversion from the present study for both free and immobilised lipase in the SMDR are higher compared to other reports using lipase from *pseudomonas.sp* for the same reaction. Also, the reported scale for this reaction to date is between 5 to 50 ml, whereas we have scaled it up to 250 ml without a loss in reaction efficiency. This indicates the potential of the SMDR for handling larger throughput compared to a batch reactor, and hence its applicability for industrial scale application of the reaction system. Fig 4.7(a) shows a small 2% increase in conversion with an increase in flowrate from 3 ml s^{-1} to 5 ml s^{-1}. This marginal increase in



(a)



(b)

Figure 4.6: (a) Comparison of reaction in batch and SMDR with free and immobilised lipase at spinning speed of 350 RPM and 3ml s^{-1} flowrate, (b) Effect of spinning speed on reaction conversion at flowrate of 3ml s^{-1}

conversion has also previously been confirmed in the SMDR and is because an increase in flowrate results in a higher contact frequency between the feed and the immobilised lipase, although it also result in a lower mean residence time between the cloth and the feed (i.e. a lower contact time per pass). The reaction rate also increased with an increase in the spinning speed comparable with the rate of the batch reaction, and a good agreement was found between the experimental and model values (Fig 4.7(b)). As can be seen from Fig 4.7(c), similar reaction rates were obtained for the reaction in batch using free lipase and the SMDR.

Table 4.2: Comparison between different amano lipases for kinetic resolution of (rac)1-phenyl ethanol

Enzyme description	**Reaction time (h)**	**Reaction volume (ml)**	**% Conversion**	**Reference**
Free PFL	12	4	28	[38]
PFL immobilised on PPU	12	4	17	[38]
Pseudomonas immobilised on diatomite	2	30	18	[12]
Amano AK-homogeneous	2	1.5	7	[6]
Lipase from PF-homogeneous batch	24	10	48	Present study
Lipase from PF-immobilised batch	24	10	30	Present study
Lipase from PF-homogeneous SMDR	5	250	26	Present study
Lipase from PF-Immobilised in SMDR	5	250	31	Present study

4.3.5 *Effect of multiple cloths in the SMDR*

The SMDR follows a concept of 'numbering up', which means that the overall catalyst loading of the system can be increased by adding more lipase cloths on the disc. From Fig 4.8(a & b), it was observed that the addition of cloths resulted in an increase in the initial reaction rate accompanied by a small increase in reaction conversion. As the cloth number increases from 1 to 3 cloths, there

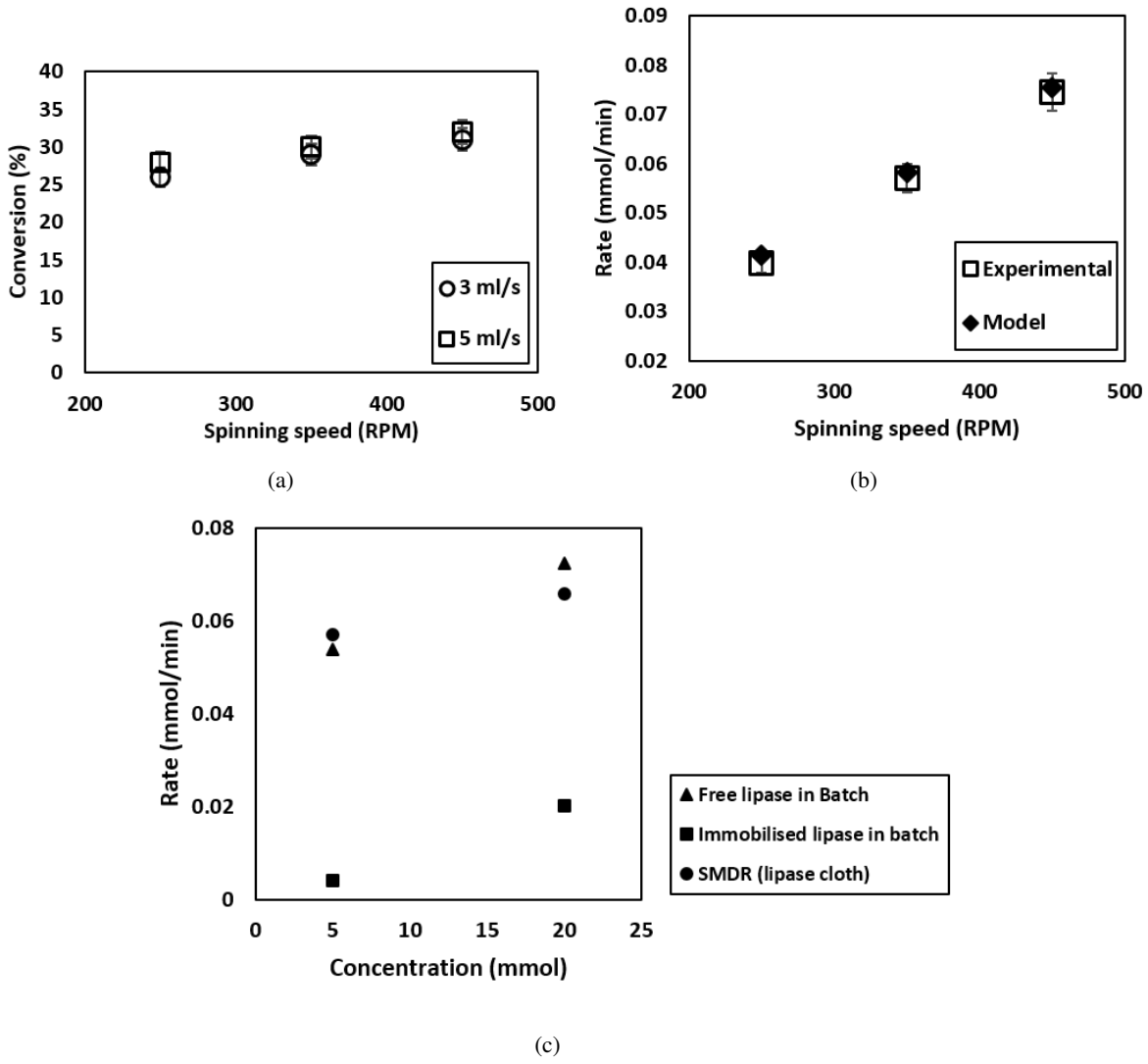
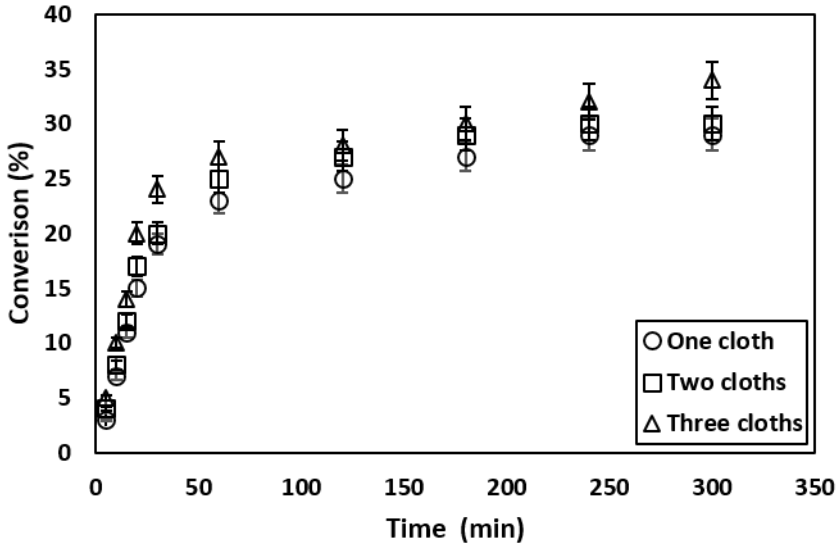
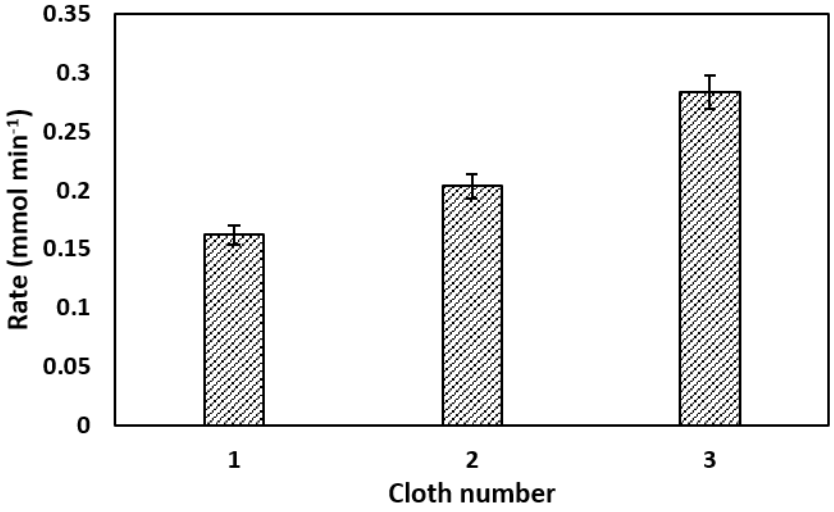


Figure 4.7: (a) Effect of flowrate on reaction conversion at different spinning speeds and (b) Experimental and model results for reaction rate and (c) Initial reaction rates in SMDR and batch reactor

is an increase in the cloth volume and enzyme loading. The increase in the surface area of the cloths is not significant compared to the increase in the cloth volume which further proves that the reaction is not solely catalysed by the enzymes bound on the outer surface but also occurs within the cloths. Also, the reaction proceeds at a faster rate for similar conversion from one cloth (0.16 mmol min^{-1}) to three cloths (0.28 mmol min^{-1}) as more number of active sites are available for a higher enzyme-substrate ratio and for a given residence time. Visual studies from the previous reports have indicated that although the centrifugal force causes the reaction liquid to flow tangentially across the disc, gravitation forces acting on the cloth stack promotes complete penetration of the reaction liquid through the cloth stack [27]. Additionally, multiple cloths on the disc surface causes an increase in the mean residence time ensuring a longer contact time between



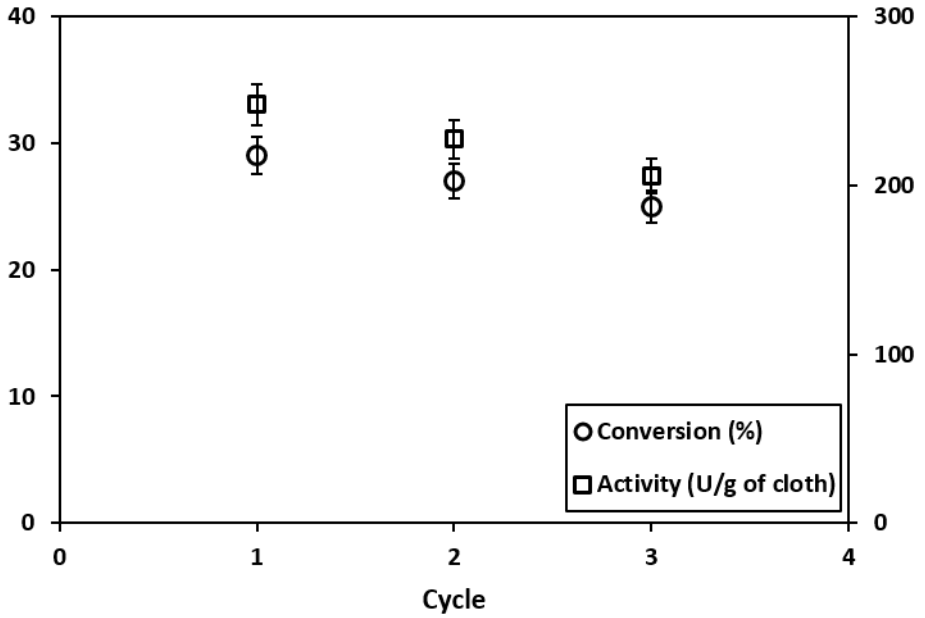
(a)



(b)

Figure 4.8: (a) The effect of number of cloths on conversion in the SMDR and (b) effect of cloth number on the initial reaction rate at spinning speed of 350 RPM and flowrate of 3 ml s^{-1}

the immobilised enzymes and the substrate. The presence of multiple cloths also causes the flow patterns to change compared to a single cloth SMDR as more flow channels are present within the fibre matrix, leading to increased mixing on and within the cloth stack [30]. The flow pattern in a conventional SDR is similar to plug flow, whereas with the addition of a cloth, the SMDR deviates from the plug flow behaviour. Addition of more clothes further reduces the number of tanks (N) in series with a wider distribution of the residence time indicating a well-mixed behaviour of the reactor. This is beneficial in the SMDR as it facilitates better contact between the substrate and the enzymes.



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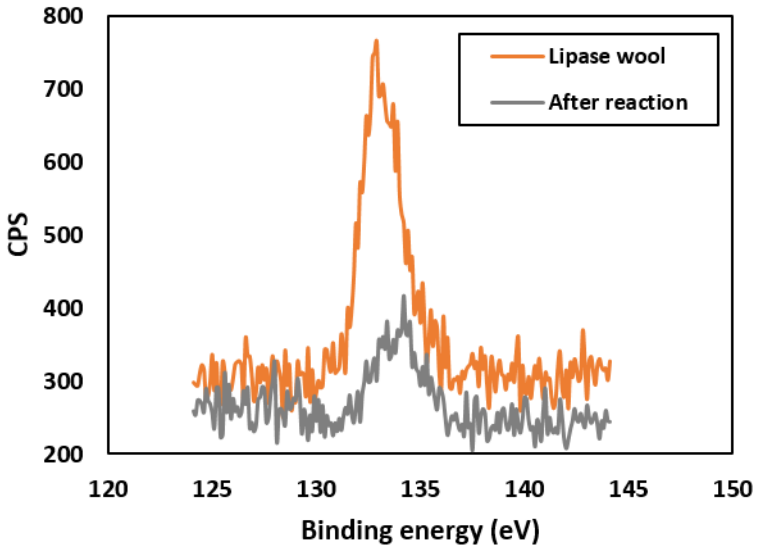

(b)

Figure 4.9: (a) Re-usability and activity of lipase cloth for multiple cycles in the SMDR at spinning speed of 350 RPM and flowrate of 3 ml s^{-1} and (b) P2p scan of used lipase wool

4.3.6 *Re-usability of lipase cloth in the SMDR*

Fig 4.9(a) demonstrates re-usability of the lipase cloths for up to 3 cycles, retaining 83% of the original activity when used in the SMDR. The loss in the activity can be due to two reasons: (1) detachment of enzymes from the cloth support; (2) enzyme deactivation caused by the reaction environment (substrate/intermediate/product). Since the reactor was operated below the critical shear stress (9500 s^{-1} as tested with ethyl acetate), only the loosely bound enzymes may have been detached from the support causing a loss in the activity.

Fig 4.9(b) shows a decrease in the concentration of phosphorous at the surface, further indicating the possibility of loosely bound enzymes leaching from the surface of wool. The resolution reaction of 1-phenylethanol is being carried out in an organic solvent medium with vinyl acetate as an acyl donor, resulting in the formation of a vinyl alcohol intermediate, followed by the product 1-phenylethyl acetate. The reaction media is characterised by the presence of alcohol functional groups, which has shown to cause enzyme deactivation (see 4.3.2), and can be one of the reasons for activity loss of the lipase cloth on re-use. Han et.al [38] have reported that the formation of acetaldehyde during the resolution reaction can inhibit the enzyme activity and selectivity, which may also be the reason for reduced activity on repeated use of lipase cloth in the present study. Other reports [11, 12] for this reaction have shown that there was little or no loss in conversion upon re-using the enzyme and this may be due to the nature of lipase or the immobilisation technique and lower agitation speeds (50 to 200 RPM) used for the reaction.

4.4 Conclusion

In this study, amano lipase from pseudomonas fluorescens was immobilised on wool and was used as a catalyst for the kinetic resolution of 1-phenylethanol. Lipase was immobilised on wool using a simple protocol and the immobilisation was verified via characterisation techniques like SEM, EDX and XPS. The effect of different solvents and temperatures were investigated on the catalyst efficiency and enantioselectivity in batch to establish optimum reaction conditions. Maximum reaction conversion was observed at 25°C in ethyl acetate and the conversion decreased with an increase in temperature for both free and immobilised lipase in batch. The reaction conversion increased with increasing spinning speed and flowrate in the SMDR due to higher mass transfer as a result of better mixing on the surface of the disc and high surface shear. The production output in the SMDR using immobilised lipase was 35% higher than batch and other conventional reactors under similar reaction conditions and the reactor successfully handled higher feed throughput. Further, the catalyst loading in the reactor was increased by simply adding more cloths on the surface of the disc and the reaction rate doubled from one cloth to three cloths. The lipase cloth was re-used for up to three cycles and 83% of the original activity was retained, demonstrating the robustness of this catalyst for organic reactions. The kinetics of the reaction in both batch and SMDR conformed well to the Ping Pong bi-bi mechanism. The overall conversion for this reaction system is comparable to that reported in literature for amano lipase catalyst and reaction intensification and scale-up was achieved in the SMDR compared to the batch reactor in the present study. This indicates potential of the reactor to carry out enzyme catalysed reactions as demonstrated through the kinetic resolution of 1-phenylethanol. The results from the study also highlight the merits of the SMDR as a process intensification technology as the reactor can support a wide range of mesh

supports and catalysts, and scaled-up by adding more mesh cloths immobilised with the catalyst. Future work is required to further optimise the reaction to achieve higher conversion through altering the support and catalyst for better compatibility with polar solvents.

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Chemo-enzymatic kinetic resolution of Henry reaction

As demonstrated in the previous chapters, the SMDR has shown potential for scaling-up organic reactions namely, nitroaldol condensation reaction catalysed by copper triflate immobilised on wool and kinetic resolution of 1-phenylethanol catalysed by lipase immobilised on wool. These reactions also form a precursor for the dynamic kinetic resolution (DKR) reaction where the product of the Henry reaction can undergo kinetic resolution catalysed by an enzyme in a single step.

4.5 Background

One-pot, cascade reactions have the potential to be the greener and a more sustainable alternative for production of chemicals. The increasing research interest in cascade reactions is due to the potential reduction in the number of reaction steps and elimination of intermediate product purification, catalyst recovery, resulting in better control over chemical equilibrium and reduced energy consumption [1]. To effect a one pot reaction in tandem, it is important to design a suitable catalyst system and identify a reactor design which can support reactions in tandem. Since many catalysts are not compatible with each other, catalyst compartmentalisation and site isolated catalysts have gained increasing attention over the last decade. SMDR has the potential to augment the cascade chemistry as site isolation of catalysts can be achieved by immobilising them on different supports.

Dynamic Kinetic Resolution (DKR) is a reaction wherein a pure racemic mixture can be converted to enantiopure compound. The reaction involves differentiating two enantiomers by the rate at which each reacts under the influence of a chiral catalyst or a reagent. Asymmetric Henry reaction coupled with kinetic resolution is a new approach towards preparing pure enantiomers (Fig 4.10). Metal and enzyme catalysts on their own exhibit various shortcomings for Henry reaction. One novel way of overcoming this issue is to use a combined metal catalyst system for asymmetric Henry reaction followed by kinetic resolution to produce optically pure compounds in one-pot. William's group [2] reported the use of ruthenium metal complex as the racemisation catalyst for asymmetric Henry reaction and Pseudomonas fluorescens (lipase) for DKR of alcohol. A conversion of 76% and an enantiomeric excess of 80% was reported. An improved protocol was developed by the Backvall group [3], who used immobilised lipase, Novozyme-435 and dimeric ruthenium complex and achieved 100% conversion and high enantioselectivities of >99.5%. The

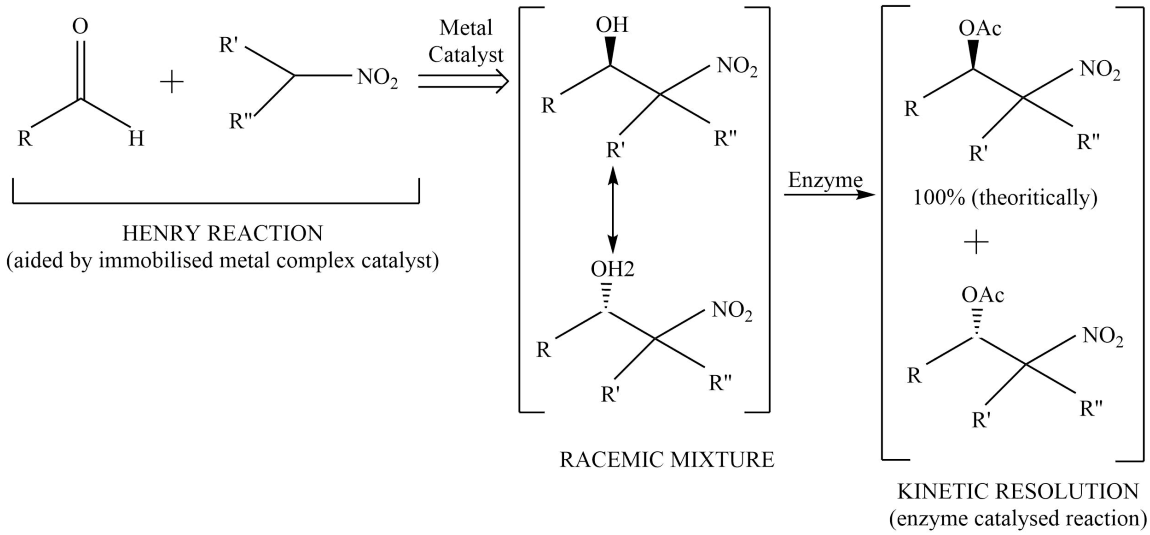

Figure 4.10: Mechanism for dynamic kinetic resolution of Henry reaction

drawback of this method was the stoichiometric amount of ketone required as a hydrogen mediator. Kim and co-workers [4] demonstrated the use of ruthenium catalyst for the racemisation of alcohol. The reaction was carried out using lipase at room temperature. High yield and selectivity of >99% was obtained. Though the reaction time for DKR was prolonged (1.3 to 7 days), the most important outcome of this work was the utilisation of the chemoenzyme catalyst for one-pot DKR at ambient conditions for very good enantioselectivity.

4.6 Results and discussion

The two steps of the reaction were first carried out individually in batch before scaling it up in the SMDR and main results have been discussed. The Henry reaction product refers to the pure product from Henry reaction which was carried out in batch using copper cloth catalyst as mentioned in chapter 2.

4.6.1 *Resolution of Henry product using a chemo catalyst*

This step was carried out to check if the Henry product could undergo resolution. The product from Henry reaction was dissolved in dichloromethane (10 ml), to which acetic anhydride (0.48 ml) and magnesium bromide (0.1 g) were added and stirred at 25°C for 48 hours in a reaction carousel. The formation of 2-nitro-1-phenylethyl acetate, with a conversion of 65% was confirmed from ^{1}H NMR.

4.6.2 *Resolution of Henry product using lipase catalyst*

In the two step process, Henry reaction was first carried out in batch on the same scale as mentioned in chapter 2 with ethyl acetate as the solvent. The solvent was changed from ethanol to ethyl acetate as it was the best solvent for both reactions as the lipase deactivated in the presence of ethanol (as seen in earlier in the chapter). The conversion of Henry reaction was found to be 72%. The copper catalyst was separated and the resolution step was carried out by adding 100 mg of lipase cloth and 0.55 ml of vinyl acetate (acylating agent) and the reaction was carried out for 24 hours. A conversion of 63% was obtained for the resolution product 2-nitro-1-phenylehtyl acetate.

4.6.3 *One pot DKR reaction in batch*

The one pot DKR of Henry reaction in batch was carried out by stirring 100 mg copper cloth and 100 mg lipase cloth with benzaldehyde (0.1 mL), nitromethane (0.55 mL), vinyl acetate (0.55 mL), triethylamine (0.016 mL) and 10 mL of ethyl acetate. The reaction was carried out for 48 hours at 25°C and monitored using NMR. A broad shift was observed at 6.3 ppm instead of the characteristic doublet of doublet for the resolution product. Also, only 21% of benzaldehyde was consumed at the end of the reaction. There can be two reasons for the unsuccessful one pot reaction. The broad peak indicates a possibility of the acylated product undergoing a side reaction forming hydrogen bonds with the solvent. Catalyst deactivation can be another reason and this was further investigated. The conversion for Henry reaction drastically dropped to 26% with copper wool catalyst in the presence of the acylating agent vinyl acetate. The reduction in the conversion was also observed when a different acylating agent like acetic anhydride was used. Hence, the

Table 4.3: Summary of results for DKR reaction in batch

Reaction	**Catalyst)**	**Conversion(%)**
Henry reaction (DKR step 1)	Copper wool	72
KR of Henry reaction (DKR step 2)	Lipase wool	63
One pot DKR	Copper and lipase wool	21
Henry reaction with vinyl acetate	Copper wool	26

one pot reaction was limited by the production of the 2-nitro-phenyl ethanol (Henry product) due to deactivation of the copper triflate catalyst in the presence of the acylating agent. Although the reaction was successful in two steps both in batch and SMDR, it is important to maintain a suitable reaction environment and equilibrium to achieve a successful one-pot reaction.

The reaction was not investigated with a different catalyst and reaction medium as it is beyond the scope of this thesis and hence not tested further in the SMDR. The potential for further reaction scale-up in the SMDR was studied using a newly designed reactor with varying disc diameter using the already optimised reactions.

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Chapter 5

Routes to Spinning Mesh Disc Reactor (SMDR) scale-up

Traditional reactors like batch and stirred tank reactors have for long been employed in the chemical industries as they are easy to operate and scaled-up by simply increasing the volume of the reactor [1]. However, this leads to the need to use large amounts of catalyst and high heat and mass transfer resistance reducing the overall efficiency of the process. Adding reactors in series or parallel improves the efficiency at every stage but this requires a large amount inventory increasing the overall production costs [1].

The catalyst loading in the SMDR has shown potential to be scaled-up by adding more catalyst cloths without the need for a complete redesign of the reactor as seen in Chapter 4. Another way to achieve scale-up in SMDR but not researched till date is by increasing the cloth size. In a conventional SDR, an increase in reaction rate has been observed with increasing disc radius for equivalent residence time [2]. This is due to the higher shear force produced by a larger disc, further improving mixing in the reactor. Similarly, in an SMDR, a larger cloth diameter results in greater catalyst loading and also higher surface shear improving the overall reaction rate.

This paper presents the comparison between two different routes for SMDR scale-up by increasing the cloth size and the cloth number in a newly designed reactor. Tributyrin hydrolysis and Henry reaction were chosen to characterise the reactor performance as they had previously been optimised in the reactor. Additional plots for this chapter can be found in Appendix C.

<table>
<tr><td colspan="9">This declaration concerns the article entitled:</td></tr>
<tr><td colspan="9">Routes to Spinning Mesh Disc Reactor (SMDR) scale-up: Investigating the effect of increasing cloth size and cloth number on reactor performance</td></tr>
<tr><td colspan="9">Publication status (tick one)</td></tr>
<tr><td>draft manuscript</td><td></td><td>Submitted</td><td></td><td>In review</td><td>✓</td><td>Accepted</td><td></td><td>Published</td></tr>
<tr><td>Publication details (reference)</td><td colspan="8">Parimala Shivaprasad, Matthew David Jones, Paul Frith, Emma Anna Carolina Emanuelsson – Under review in Chemical Engineering and processing: Process Intensification journal.</td></tr>
<tr><td>Candidate's contribution to the paper (detailed, and also given as a percentage).</td><td colspan="8">Formulation of ideas (75%): The co-authors contributed to the reaction schemes discussed in this paper. The reactor design and development was carried out by Paul Frith. I contributed to the analysis and reaction optimisation study presented in the paper

Design of methodology (80%): I planned the design of experiments for optimisation and Emma Emanuelsson contributed to the additional experiment plan to justify the findings presented in this paper.

Experimental work (95%): I performed all the experiments and carried out most of the data interpretation after subsequent discussions with Emma Emanuelsson.

Presentation of data in journal format (90%): I prepared the manuscript including graphics in journal format, data interpretation and incorporating feedback from the co-authors. Emma Emanuelsson contributed to improving individual sections by providing feedback during the manuscript preparation stage.</td></tr>
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Abstract

The spinning mesh disc reactor (SMDR) is a rotating catalytic reactor with a potential to facilitate process intensification. In this study, the scale-up of a newly designed SMDR has been demonstrated by increasing (i) cloth size and (ii) cloth number for tributyrin hydrolysis and nitroaldol condensation reaction. The effect of spinning speed, cloth size and cloth number was investigated using design of experiments and the results show an increase in the cloth size or cloth number leads to a higher reaction rate. This is due to (i) an increased enzyme loading with increase in surface area and volume of the cloth stack and (ii) reduced film thickness with increasing shear forces and longer residence times improving the overall mass transfer. Addition of multiple cloths of increasing cloth sizes further improved the reaction rates at higher substrate concentration. A maximum reaction rate of 6.9 mM min^{-1} and 0.043 mmol min^{-1} was obtained for three 50 cm cloths for tributyrin hydrolysis and nitroaldol condensation reaction respectively. These results highlight the potential routes for the SMDR scale-up without a loss in the reaction efficiency for aqueous and organic reaction systems, thus allowing for a tuneable operation of the SMDR for industrial applications.

5.1 Introduction

Scale-up of traditional reactors is straightforward but accompanied by high mass and heat transfer resistance impeding the reaction rate and reduced product yield. Hence, careful consideration of the reaction system is necessary to ensure a sustainable process scale-up as they often involve large quantities of solvents and expensive catalysts. This can be overcome by achieving a better control over the reaction at a molecular level and follows a new development path in chemical processing known as process intensification (PI) [3]. PI can be achieved either by modifying the reactor design or employing innovative reaction techniques. One of the more popular approaches of PI is to achieve a reduction in the size of the chemical equipment or simplify its scale-up whilst maintaining the production efficiency [4]. This has led to the concept of 'numbering-up', where process scale-up is achieved by adding more reactor units in series or parallel. Generally, numbering-up reduces the reactor volume which is beneficial for carrying out hazardous reactions alleviating the need for large amounts of chemicals and hence promoting safe operating conditions.

The spinning disc reactor (SDR) is an intensified reactor which uses centrifugal force to drive the reaction fluid to a thin film of high shear on the surface of the spinning disc [5]. Scale-up in the SDR can be achieved by either increasing the disc size or by adding more discs ('numbering-up') to the central shaft. Scale-up by increasing the disc size in the SDR has been achieved for production of polyurethane [2]. A modification of the SDR is the rotor stator spinning disc reactor (rs-SDR), where it has been shown that addition of multiple rotors to the central axis results in improved mass and heat transfer co-efficient for multi-phase and catalytic reactions [6, 7]. The spinning mesh disc reactor (SMDR) is another variation of the SDR, which additionally houses a mesh cloth support with immobilised catalyst on the disc. The reaction liquid impinged on the centre of the spinning disc allows for the formation of a thin film of high shear over and within the cloth surface, facilitating rapid mixing and enhanced mass transfer. Further, residence time distribution studies has shown that addition of cloths causes a deviation from the plug flow behaviour reported for the SDR and increases the well-mixed behaviour of the SMDR [8].

Reaction intensification has been demonstrated by using catalysts immobilised on the wool for: (i) lipase catalysed hydrolysis of tributyrin [9], (ii) Henry reaction catalysed by copper triflate [10] and (iii) kinetic resolution of racemic alcohol catalysed by amano lipase [11] and improved reaction efficiency was achieved in all the cases compared to the batch reactor. Process scale-up in the SMDR has been achieved through the concept of 'numbering up' by addition of more catalyst cloths on to the disc. Significant improvement in the reaction rate has been observed for the systems described above with the increase in the number of catalyst cloths. This demonstrates that the transition of SMDR from a laboratory process to an industrial scale operation is accompanied by minimum alterations to the original reactor design without compromising on the overall process

efficiency. However, the potential to scale-up the SMDR by increasing the diameter of cloths and numbering up larger cloths has not yet been explored.

The aim of this study is thus to investigate and compare the performance of the SMDR when scale-up is achieved by: (i) increasing the catalyst cloth size and (ii) addition of multiple cloths. Two different reactions: enzymatic hydrolysis of tributyrin (aqueous system) and nitroaldol condensation reaction catalysed by copper triflate (organic solvent system) have been chosen for this study. The reactions will be carried out in a newly designed reactor with an optimised nozzle design to minimise the spin-up zone and also improve the contact between the reactant and the cloth surface. The effect of increasing cloth diameter (20 cm to 50 cm) and number of cloths (one to three) on the reaction rate will be investigated using design of experiments. The reactor performance will also be examined as a function of film thickness and mean residence time.

5.2 Material and methods

5.2.1 *Materials*

All chemicals and lipase from *Pseudomonas fluorescens* was obtained from Sigma Aldrich and used as received. Unbleached wool was obtained from Urbanara (Berlin, Germany). Deionised water was used to prepare all standard solutions and buffers.

5.2.2 *Immobilisation of lipase on wool*

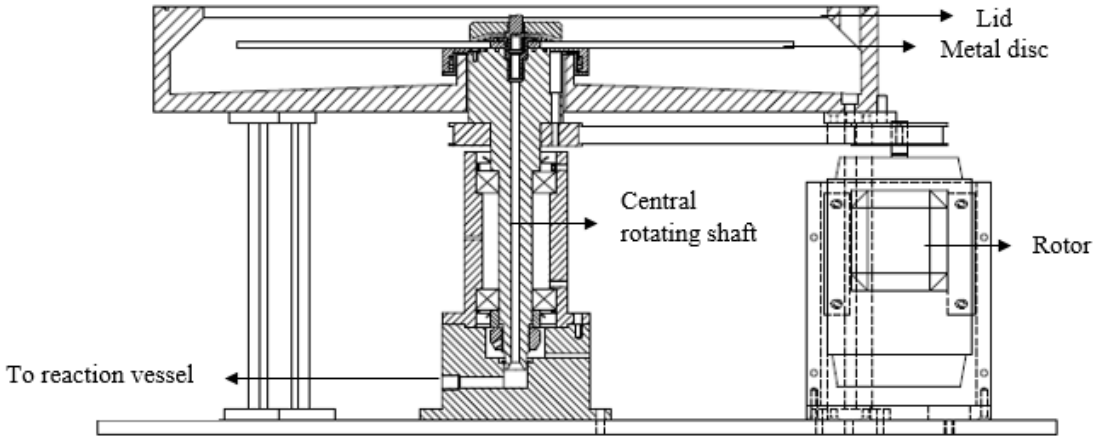
The immobilisation of lipase on wool has been explained in detail in our previous publications [9]. To summarise, circular pieces of different diameters (20 cm, 30 cm and 50 cm) were cut from a woollen blanket cloth. Pre-treatment of the cloths was carried out using a solution of sodium silicate and hydrogen peroxide in a pH 9 buffer for 70 minutes. The cloths were then soaked in a solution of 2% polyethylene imine (PEI) at pH 8 for 2 hours and washed with deionised water, after which they were soaked in a solution containing 2 gL^{-1} lipase in a pH 6 phosphate buffer for 24 hours. Finally, the cloths were cross-linked in a 0.5% glutaraldehyde solution in pH 6 phosphate buffer for 10 minutes.

5.2.3 *Copper triflate immobilisation on wool*

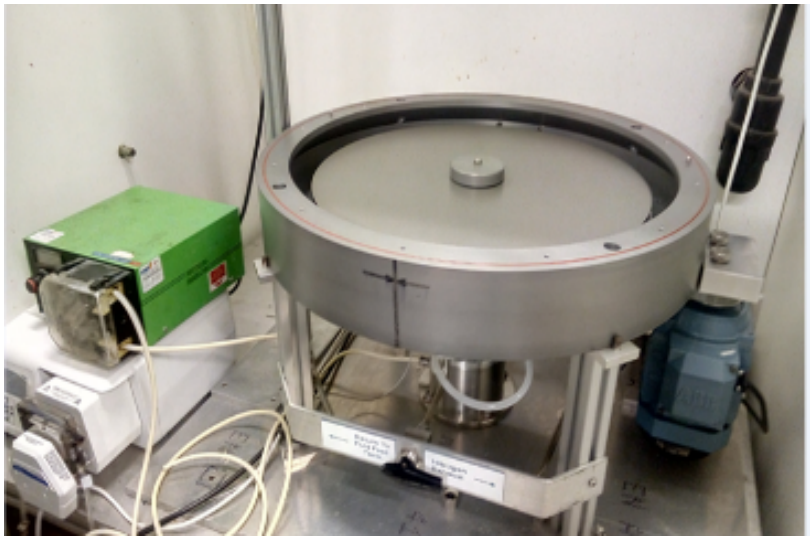
The detailed protocol has been published in our previous publication [10]. The procedure for copper immobilisation is similar to that of lipase on wool. The woollen cloths after the PEI treatment were soaked in copper triflate solution (1 mM) in methanol for 24 hours. The crosslinking of copper on wool was carried out in a similar manner to that of lipase cloths.

5.2.4 *Reactions in the SMDR*

As shown in 5.1(a), the SMDR consists of a circular disc fastened with the catalyst cloth and connected to a central rotating shaft within a circular housing. The spinning speed is controlled via a variable speed controller and the disc is driven by a three phase AC motor. The inlet reaction



(a)



(b)

Figure 5.1: (a) Schematic diagram and (b) photograph of the SMDR

liquid from the reactant vessel is pumped through the central rotating shaft and through a nozzle located at the centre of the disc. The liquid at the centre of the disc has the same speed as the spinning disc, hence minimising the spin-up zone from the centre of the disc when the reactant comes in contact with the disc. This maximises the area of contact between the cloth and the reactant, ensuring a uniform coverage of the cloth. The spin-off from the edge of the disc passes through circular ports located around the casing and into a reservoir from where it is pumped back

into the reaction vessel. The disc and the housing are made of hard anodised aluminium and the inlet port tubing is stainless steel for better solvent resistance.

(a) **Tributyrin hydrolysis**

An emulsion of tributyrin was prepared by adding triton X-100 and tributyrin to 1000 ml of pH 7 phosphate buffer (0.1M) such that the final concentration of the reagents were 3.5 gL^{-1} and 33 mM respectively. The mixture was emulsified at 1000 RPM for 10 minutes. Lipase cloth

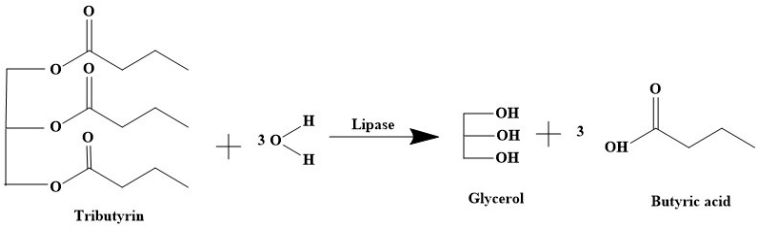


Figure 5.2: Lipase catalysed hydrolysis of tributyrin

of the desired size was fastened to the disc and rotated at a set rotation speed. The reaction mixture was fed to the reactor via a peristaltic pump. The reaction was run for 3 hours at 45°C. A pH stat was used for continuous addition of sodium hydroxide to maintain a constant pH and the data was recorded continuously. The reaction conversion was determined as follows [9]:

$$\text{Conversion}(\%) = \frac{\text{moles of free butyric acid}}{\text{moles of original esters in tributyrin}} \times 100 \tag{5.1}$$

The initial reaction rate was calculated from the slope of the hydrolysis curve obtained from the pH stat. All data points were used from the start of the reaction till the slope began to decrease, which indicates the end of the initial rate period [12].

(b) **Nitroaldol condensation reaction**

The reaction mixture consisting of benzylamine (1 mmol), trimethylamine (0.16 mmol), nitromethane (10 mmol) and dodecane (0. 5mmol) was dissolved in 500 ml ethanol. The copper catalyst cloth was fastened to the disc in the reactor and the reactor was covered with a Perspex lid. The reaction was initiated by switching on the peristaltic pump once the reactor reached the set rotation speed. The reaction was carried out at 25°C for 3 hours. Samples were taken every 5 minutes during the first 30 minutes of the reaction and thereafter for every hour till the end of 3 hours. The samples were analysed using gas chromatography (GC) using a method described in our earlier publication [10]. All experiments have been carried out in triplicates with a degree of confidence of 95%.The initial reaction rate was calculated for the first 60 minutes of the reaction time period using 1^{st} order rate kinetics as follows:

$$r = -\frac{d[C]}{dt} = k.[C] \tag{5.2}$$

The integration of the above equation between the initial and final benzylamine concentration results in the following equation:

$$-ln\frac{[C]}{[C_0]} = k.t \tag{5.3}$$

Where, r is the reaction rate (mmol min^{-1}) and k is the first order reaction constant (min^{-1}) and t is the reaction time (min).

The initial reaction rate was calculated by multiplying k by the initial benzylamine concentration.

The average surface shear ($\bar{S}$) was used to characterise the reactor performance based on the cloth size and the spinning speed for both reactions as follows [9]:

$$\bar{S} = \frac{1}{R}\int_0^R Sdr = \frac{3}{4}\left(\frac{3QR\omega^4}{2\pi v^2}\right)^{1/3} \tag{5.4}$$

Where, $\bar{S}$ = shear stress (s^{-1}); Q = Volumetric flow rate (m^3s^{-1}); R = Radial distance (m); ω = Angular velocity (rad s^{-1}); ν = Kinematic viscosity (m^2 s^{-1}).

The resulting film thickness (f) as a function of the spinning speed was calculated using the following equation [2]:

$$f = \left(\frac{3vQ)}{2\pi\rho R^2\omega^2}\right)^{1/3} \tag{5.5}$$

where, f = film thickness (m); μ = liquid viscosity (Pa s); Q = volumetric flow rate (m^{-3} s^{-1}); ρ = liquid density (kg m^{-3}); R = radius of the disc (m); ω = angular velocity (rad s^{-1}).

The mean residence time (t) of the liquid feed on the cloth surface was calculated as follows [2]:

$$t = \left(\frac{81\pi^2 v}{16\rho Q^2\omega^2}\right)^{1/3} R^{4/3} \tag{5.6}$$

Where, t = mean residence time (s); R = radius of the disc (m); μ = liquid viscosity (Pa s); ρ = liquid density (kg m^{-3}); Q = volumetric flow rate (m^3 s^{-1}); ω = angular velocity (rad s^{-1}).

5.2.5 *Design of Experiments (DOE)*

The effect of different factors such as spinning speed, cloth number and cloth size on reaction conversion and rate was analysed using the Design of Experiments (DoE) approach. Minitab statistical software was used to create a two-level full factorial design and the experiments were performed in random order. The response as a function of conversion and rate were analysed using a normal and a Pareto chart plot. The standardised effect of the factors were plotted according to their order of significance in the Pareto chart and the dotted line indicating a significance of 5%. The interaction plots were used to determine the effect of interacting variables on the response, with converging lines indicating significant influence. The significance of the variables have been predicted with a degree of confidancel of 95%. The software is able to carry out error analysis by using a statistical model to estimate hoe close the actual results fit with the model, hence eliminating the need to repeat experiments.

5.3 Results and discussion

5.3.1 *Effect of increasing cloth size and cloth number on the enzymatic hydrolysis of tributyrin in the SMDR*

The effect of different cloth size and cloth numbers were examined on reaction conversion and rate of tributyrin hydrolysis in the SMDR.

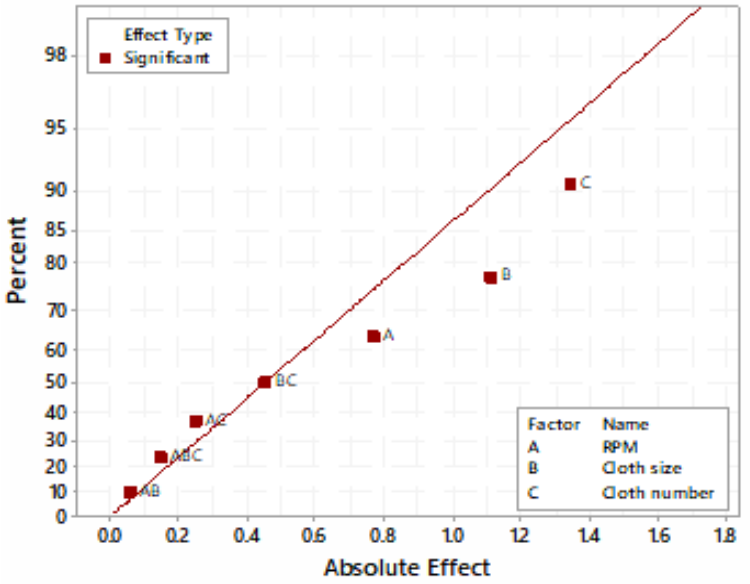
Design of Experiment: A two-level full factorial design was created using Minitab statistical software to analyse the interaction of spinning speed, cloth size and cloth number on reaction conversion and rate. The results from the experiments are summarised in Table 5.1 and discussed with respect to the initial reaction rate, as the response as it incorporates more data points during the course of the reaction compared to the reaction conversion, which is a single end point value. The hydrolysis reaction occurs in two steps. The first faster stage is due to the hydrolysis of the first ester bond in the water phase and available readily for the lipase. The reaction rate decreases thereafter due to the steric hindrance caused by the remaining ester bonds that are not accessible to the lipase. During the course of the reaction, the reaction solution turns from a cloudy emulsion to a clear solution. The tributyrin emulsion along with Triton-X and lipid/glyceride can form micelles dispersed in the aqueous phase and these micelles are optically clear [14, 15]. The oil droplets and the micelles co-exist at the beginning of the reaction and as hydrolysis proceeds, the number of oil droplets decrease resulting in a greater proportion of hydrophilic micelles, with the reaction solution gradually turning clear [9]. Hence, the reaction rate has been calculated only for the first faster stage to better understand the effect of changing reactor parameters on the scale-up. As can

Table 5.1: Results from the Design of Experiments studies for tributyrin hydrolysis

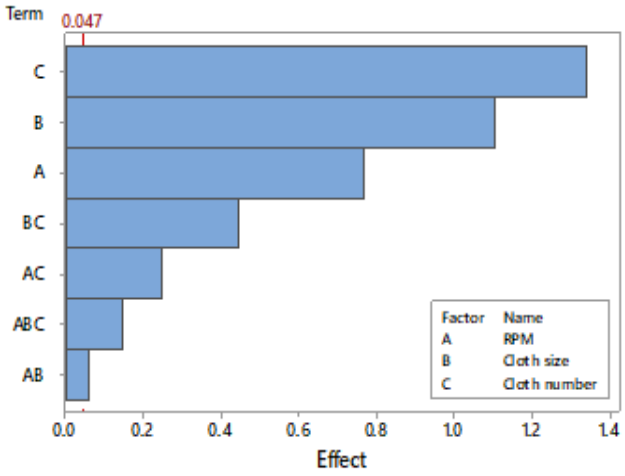
Entry	**Spinning speed (RPM)**	**Cloth size (m)**	**Cloth number**	**Initial rate (mM min^{-1})**	**Conversion (%)**
1	450	0.5	1	5.03	87
2	250	0.2	1	2.46	67
3	450	0.2	1	3.56	75
4	250	0.5	1	4.10	83
5	250	0.5	3	5.10	85
6	450	0.2	3	4.96	82
7	450	0.5	3	5.82	91
8	250	0.2	3	4.64	79

be seen from Fig 5.2 (a) and (b), the normalised effects plot and the Pareto plots for reaction rate

as the response indicates that cloth number, cloth size and RPM (in this order) have a significant effect on the conversion. In the effects plot, the factors that are farthest away from the origin have



(a)



(b)

Figure 5.3: DoE plots with reaction rate as response: (a) Effects plot, (b) Pareto chart plot

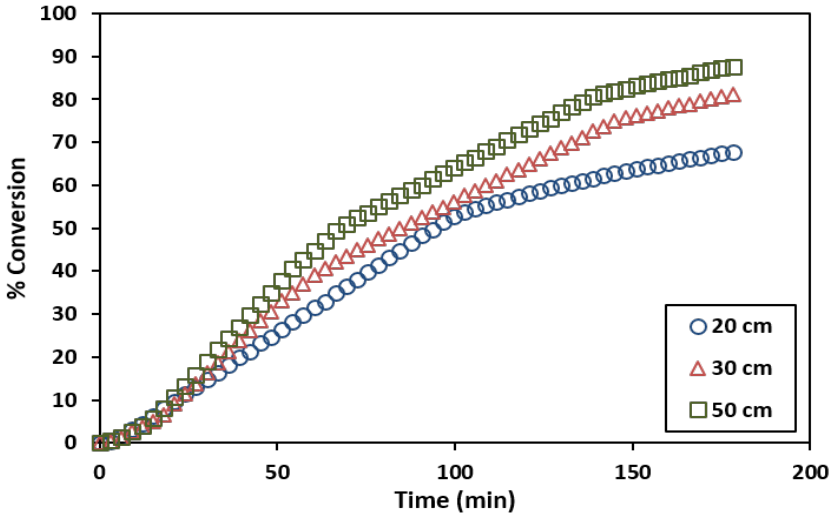
a greater significance on the response. Increasing cloth number and cloth size individually has a greater effect on the reaction rate compared to increasing both variables at the same time. This will be discussed further in the following section. Spinning speed on its own also has a positive effect on the reaction rate but less significant compared to cloth size and number. This is because the enzymatic reaction is operated at a spinning speed which corresponds to that below the critical shear stress to minimise enzyme deactivation [9, 10], hence capping the upper limit of the spinning speed to 450 RPM. Also, the interaction effect of variables does not have a significant impact on the reaction rate. Overall, the results from the DoE studies indicates that reaction scale-up in

the SMDR primarily depends on either the cloth size and cloth number and increasing multiple factors at the same time does not have a significant effect on the reaction rate for a given substrate concentration.

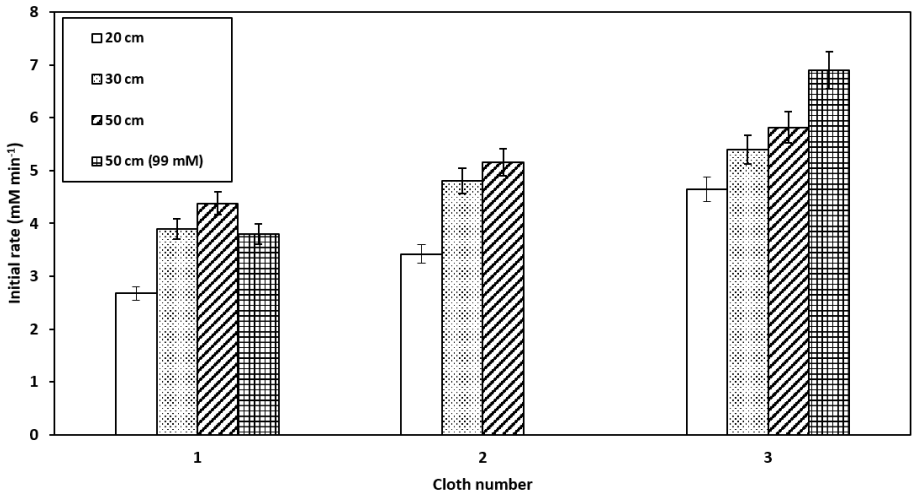
Effect of cloth size: An increase in the cloth size results in an increase in the overall surface area of the cloth and also the catalyst loading for a given substrate concentration. Fig 5.3(a) shows an increase in the reaction conversion from 67.6% to 87.7% after 3 hours and Fig 5.3(b) shows an increase in the reaction rate from 2.67 mM min^{-1} to 4.64 mM min^{-1} with an increase in the cloth size from 20 cm to 50 cm. As can be seen from Fig 5.4(a), an increase in the cloth radius results in a lower mean film thickness due to increasing surface shear (equation 5.3). This results in rapid mixing within the thin film of liquid over the cloth surface resulting in lower mass transfer resistance. Additionally, it was also found that the mean residence time increased with cloth size (Appendix C) affording better contact with the substrate and the catalyst. Hence, the mean film thickness and residence time are additional factors facilitating higher reaction rates with an increase in the cloth size.

Numbering up of cloths: Fig 5.3(b) shows the effect of numbering up catalyst cloth on reaction rate and the reaction rate increased with addition of cloths across the various cloth sizes. The reaction rate for three 20 cm cloths (4.64 mM min^{-1}) is comparable to the reaction rate obtained using one 50 cm cloth (4.38 mM min^{-1}). Addition of cloths results in an increase in the total volume of the cloth stack and hence the enzyme loading (Appendix C). Visual observations have shown that the centrifugal and gravitation forces promote complete wettability of the cloth stack in both tangential and downward direction facilitating reaction both on and within the catalyst cloth surface [13]. The increase in the reaction rate for multiple 20 cm cloths is more pronounced compared to multiple 30 cm and 50 cm cloths at a lower substrate concentration. This is because, the catalyst:substrate ratio is high even with a single large cloth and the reaction goes to completion at a faster rate and no further increase in the reaction rate is observed with the addition of lipase cloths. In difference, when the concentration of tributyrin was increased to 99 mM (i.e. decreasing the catalyst: substrate ratio) a significant positive effect can be observed for the 50 cm catalyst cloth. The rate increased from 3.8 mM min^{-1} (one cloth) to 6.96 mM min^{-1} (three cloths). At this higher substrate concentration, the reaction rate is limited by the number of active sites present on the cloth. Hence, further showing that the reaction rate, and the productivity can be tailored by increasing both the cloth size and cloth number at higher substrate concentration.

Fig. 5.4(b) shows the increase in mean residence time with increasing cloth size and number. The mean residence time for a 50 cm cloth is nearly twice that of three 20 cm cloths. Addition of cloths in the SMDR leads to an increased resistance to the flow by the cloth fibre mesh creating



(a)

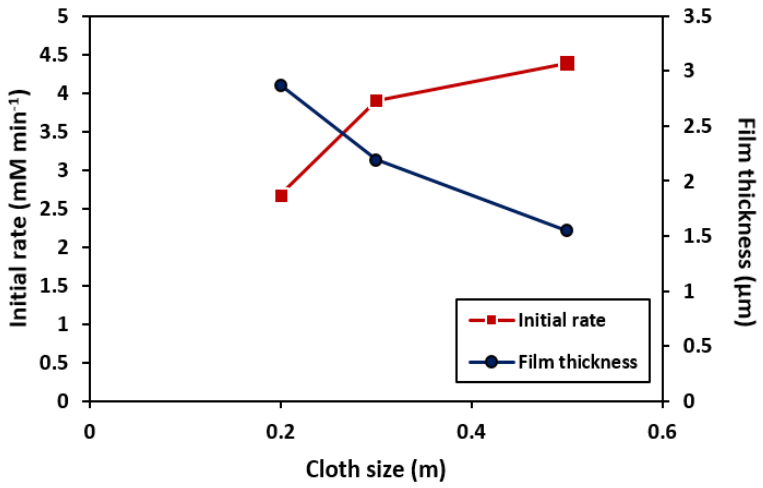


(b)

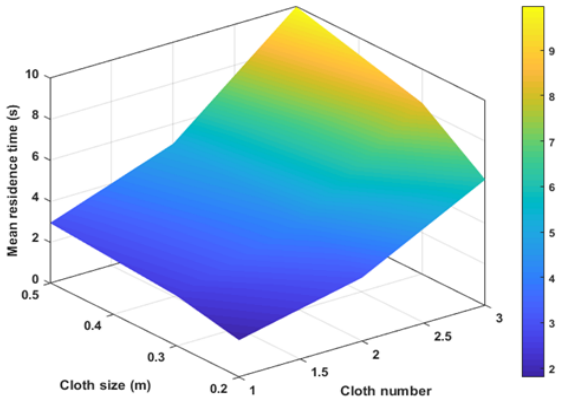
Figure 5.4: (a) Effect of cloth size on reaction conversion as a function of time for flowrate of 3 ml s^{-1} and 33 mM substrate concentration, (b) Comparison of initial reaction rates for different cloth size and cloth numbers. The experiements were carried out at 450 RPM and a flowrate of 3 ml s^{-1}. The conversion plot for 99mM can be found in Appendix C.

multiple flow channels resulting in a well-mixed reactor compared to a conventional spinning disc reactor which follows plug flow behaviour.

These results indicate that the scale-up in the SMDR can be achieved by either increasing the cloth size or cloth number. The increasing reaction rate with increasing cloth size is primarily due to the increase in catalyst loading and the mean residence time. A further increase in the reaction rate can be achieved by numbering up the catalyst cloths of increasing diameters facilitating a tuneable operation of the SMDR.



(a)



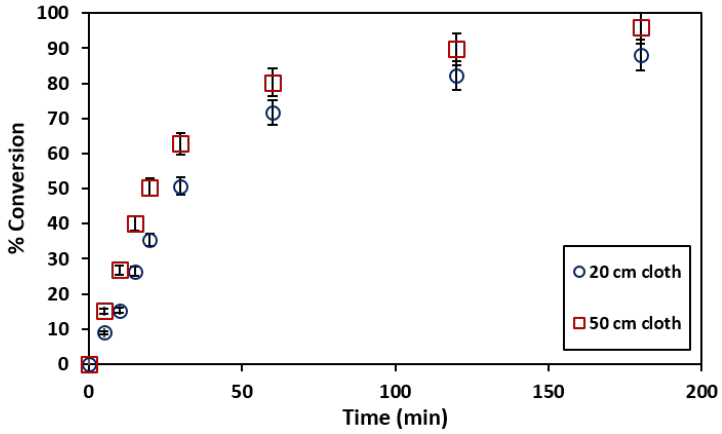
(b)

Figure 5.5: (a) Effect of film thickness on rate and (b) Mean residence time as a function of increasing cloth size and cloth number. The reactions were carried out at spinning speed of 450 RPM and flowrate of 3 ml s^{-1}

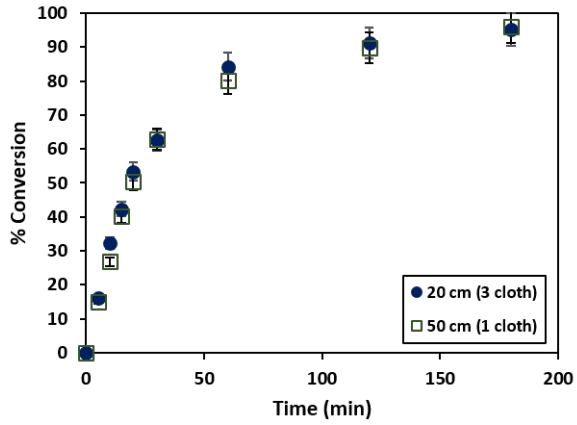
5.3.2 *Effect of changing cloth size and cloth number on nitroaldol condensation reaction in the SMDR*

To establish the proof of concept for scale-up of organic reactions in the SMDR, nitroaldol condensation reaction was carried out by altering the copper catalyst loading through increasing cloth size and cloth number.

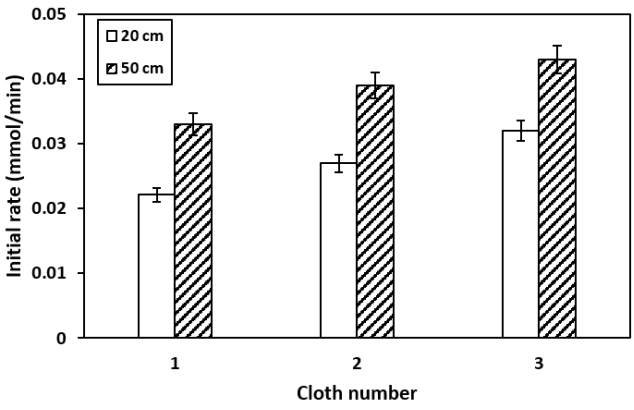
Effect of cloth size: Fig 5.5(a&b) shows the reaction conversion increased with increase in cloth size and the initial reaction rate increased from 0.018 mmol min^{-1} (20 cm cloth) to 0.033 mmol min^{-1} (50 cm cloth). Further, the average surface shear with increasing cloth size and rotation speed for the organic solvent system was nearly 1.5 times less compared to the aqueous system (Appendix C). Hence, the increase in rate for organic reactions is mainly due to the increase in the



(a)



(b)



(c)

Figure 5.6: (a) Effect of cloth size on reaction conversion as a function of time, (b) Comparison between reaction conversion for three 20 cm cloths and a 50 cm cloth and (c) Initial reaction rate comparison for scale-up by increasing cloth diameter vs increasing cloth number. All reactions were carried out at a spinning speed of 450 RPM and flowrate of 3 ml s^{-1}.

amount of catalyst immobilised with increasing cloth surface area and a longer residence time on the cloth surface. The surface shear on the cloth surface is dependent on the fluid properties of the reaction medium, which varies according to the solvent used and hence the increase in reaction

rate is less profound compared to the aqueous system.

Effect of numbering up cloths: Similar to tributyrin hydrolysis, addition of multiple 20 cm cloths (up to 3 cloths) resulted in a reaction rate similar to that obtained using a 50 cm cloth. The reaction scale-up through increasing cloth size and cloth number resulted in a maximum reaction rate of 0.043 mmol min^{-1} for three 50 cm cloths (Fig (5.5(c)). Although the liquid feed had penetrated through the entire cloth stack, a compounding effect in the reaction was not observed as seen for the aqueous system. This is due to a lower volume of liquid entrapped in the cloth stack (due to lower wettability of woollen cloth by ethanol compared to water) and hence lesser contact with the feed stream and the catalyst.

Overall, the results indicate that the SMDR can be scaled for organic reactions either by increasing the cloth size or cloth number as demonstrated for the above reaction. To the best of the authors' knowledge, this is the first report on the scale-up of nitroaldol condensation reaction in the SMDR.

5.4 Conclusion

In this study, scale-up of SMDR was investigated using two routes: (i) increasing cloth size and (ii) addition of multiple cloths. The reactor performance was evaluated for the hydrolysis of tributyrin and nitroaldol condenstation reactions as the reactions were previously optimised in the SMDR. The reactions were carried out in a newly designed reactor allowing for various disc diameters and reduced spin-up zone on the disc surface. A two level, full factorial DoE study was carried out to understand the effect of spinning speed, cloth size and cloth number on the reaction rate. Results from the DoE studies indicated that increasing either cloth size or cloth number individually had a greater effect on the reaction rate for a given substrate concentration. Further, increasing the cloth size resulted in an increase in conversion and reaction rate as a result of increased surface area of the catalyst cloth. The reaction rate achieved with a single 50 cm cloth (4.38 mM min^{-1}) was comparable to that of three 20 cm cloths (4.64 mM min^{-1}) for a tributyrin substrate concentration of 33 mM. Addition of multiple cloths of increasing cloth size also showed an increase in the reaction rate and the reaction rate increased by 36% at a substrate concentration of 99mM for three 50 cm cloths. The study was extended to nitroaldol condensation reaction catalysed by copper immobilised on wool. Similar to the previous case, an increase in reaction conversion and rate was observed with increase in cloth size and cloth number and addition of three 20 cm catalyst cloths yielded similar rate (0.032 mmol min^{-1}) to that of a single 50 cm cloth (0.033 mmol min^{-1}). Scale-up by increasing the cloth size reduces the time taken for immobilising multiple cloths and also alleviates the non-unifrom activity across different cloths. However, the overall size of the reactor increases with increasing disc size adding to the cost of equipment. Numbering

up is a more feasible scale-up option when retrofitting a reactor design and for hazardous reactions to reduce the volume of liquid hold up within the reactor. Overall, it can be concluded that the reaction scale-up in the SMDR can be achieved by increasing the cloth size and the cloth number for both aqueous and organic reaction systems, making it a flexible reactor design for reaction intensification. Future work should aim to examine higher reaction throughput for scale-up of organic reactions.

Acknowledgement

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Copper Catalysed Oxidative Amine Homocoupling in a Spinning Mesh Disc Reactor

5.5 Introduction

Imines are an essential intermediate for the synthesis of nitrogen containing and biological active compounds. Among the several reported methods for imine synthesis [1], catalytic oxidative coupling of primary amines has gained increasing research interest as the synthesis can take place under ambient to mild reaction conditions compared to other routes [1–3]. Traditionally, this reaction has been catalysed by expensive metal catalyst like palladium, gold and vanadium which makes it economically unattractive [4–6]. Hence, there is a need for an alternate catalyst which is selective to the reaction, economic and environment friendly. Copper salts and complexes [1–3, 7, 8], both homogeneous and heterogeneous, have shown potential to effectively catalyse the reaction with yield and selectivity >99% for ambient to mild reaction conditions. However, the current drawbacks are long reaction times, catalyst leaching (in case of heterogeneous catalyst) and dehydrogenation of the product resulting in reduced yield. Also, the reaction till date has only been carried out on a scale of 50 ml and hence there is an opportunity to investigate the scale-up of the reaction for industrial application.

As seen in chapter 2, the SMDR was used for intensifying Henry reaction catalysed by copper catalyst immobilised on wool [9]. This shows the potential of the reactor and the catalyst system for imine synthesis through oxidative homocoupling of a primary amine. The aim of this study is to: (i) study the catalytic property of copper triflate for oxidative homocoupling of 4-methoxybenzylamine and (ii) investigate reaction scale-up in the SMDR. The reaction will first be carried out in batch using both free and immobilised copper triflate catalyst. The scale-up in the SMDR will then be carried out using the copper catalyst cloth. The effect of spinning speed and flowrate on reaction conversion will be examined. The reaction kinetics will be presented for reactions in both batch and SMDR.

5.6 Materials and methods

5.6.1 *Materials*

4-methoxybenzylamine was obtained from Fluorochem and all other chemicals were obtained from Sigma Aldrich and used as received. All solvents were obtained from VWR international. Unbleached wool was procured from Urbanara (Berlin, Germany).

5.6.2 *Immobilisation of copper triflate on wool*

The detailed procedure of immobilisation is as mentioned in chapter 2. In summary, woollen cloth was pre-treated with PEI and soaked in copper triflate solution (1 mM) in methanol for 24 hours and cross linked with glutaraldehyde. Characterisation of the catalyst cloth can be found in our previous publication [9].

5.6.3 *Homocoupling reaction in batch*

In a typical reaction, 0.5 mmol of 4-methoxybenzylamine was stirred with of free catalyst (0.01g) or immobilised copper catalyst (1 g) in 100 ml acetonitrile at 30°C (Fig 5.7). Samples were taken

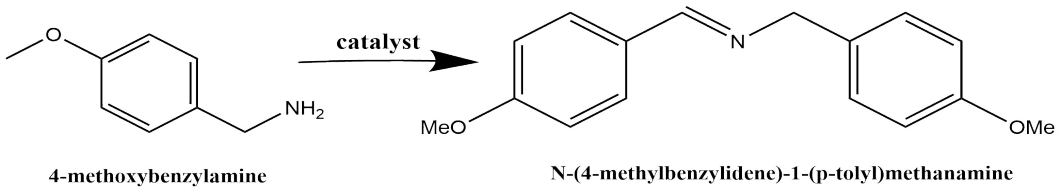


Figure 5.7: Reaction scheme for homocoupling of 4-methoxybenzylamine

at an interval of 5 minutes for upto one hour. The samples were run through a short bed of silica to remove any traces of copper and analysed using proton NMR technique using solvent suppression for acetonitrile. All experiments were carried out in triplicatres and the results have a degree of confidence of 95%.

5.6.4 *Homocoupling reaction in the SMDR*

A reaction solution containing 4-methoxybenylamine (1mmol) dissolved in 250 ml acetonitrile was used for the reaction in the SMDR. A 50 cm copper cloth was fastened onto the disc and the desired disc spinning speed was set. The reaction was initiated by starting the peristaltic pump. The reaction was carried out for 1 hour at 30°C and samples at every 5 minute interval for the first 30 minutes and every 15 minutes thereafter. The samples were analysed in a similar way as the batch reaction.

5.7 Results and discussion

5.7.1 *Homocoupling of 4-methoxybenzylamine in batch*

The reaction in batch was carried out using both free and immobilised copper triflate and a maximum conversion of 70% and 42% was obtained after respectively (Fig 5.8). The reduction in the

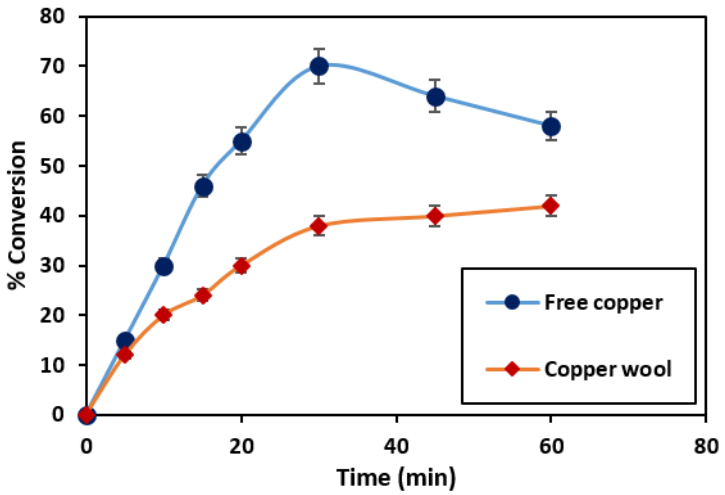


Figure 5.8: Reaction conversion using free and immobilised catalyst in batch (rotation speed 500 RPM)

reaction conversion using copper wool is due to the diffusion resistance offered by the liquid film surrounding the catalyst cloth, the reactants have to overcome from the bulk of the solution. The reaction conversion using free copper triflate deceased after 30 minutes. The reduction in conversion may be due to the decomposition of the product N-(4-methylbenzylidene)-1-(p-tolyl)methanamine into benzaldehyde as reported by Jones et.al [1]. This was not the case when the reaction was carried out with copper wool. Copper triflate is bound to wool via polyethylene imine (PEI) and the resulting complex may have a limiting effect on product decomposition. Catalyst support materials have shown to have an effect on the product degradation in similar reaction systems [1]. To test this, a control reaction was carried out with plain wool but no reaction occurred. Hence, better reaction yields are immobilised copper triflate compared to the free catalyst. The increase in catalyst and amine concentration resulted in a marginal increase in the reaction yield (Fig 5.9).

5.7.2 *Homocoupling of 4-methoxybenzylamine in the SMDR*

The reaction was carried out in the SMDR using the copper cloth at the catalyst. The reaction conversion increased from 45% to 56% with an increase in spinning speed from 250 to 450 RPM (Fig 5.10(a)). Previous reports have shown that an increase in spinning speed allows for the formation of thin film of increasing surface shear over and within the cloth surface, creating a zone of rapid

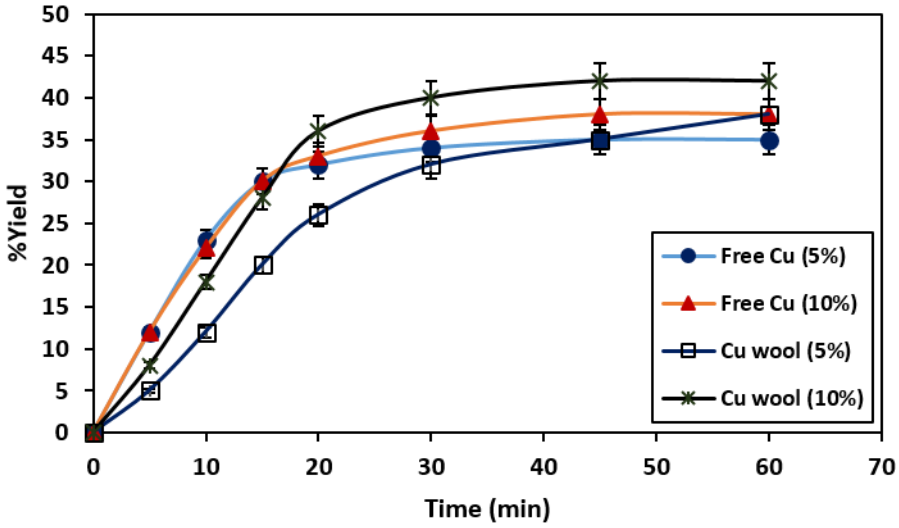


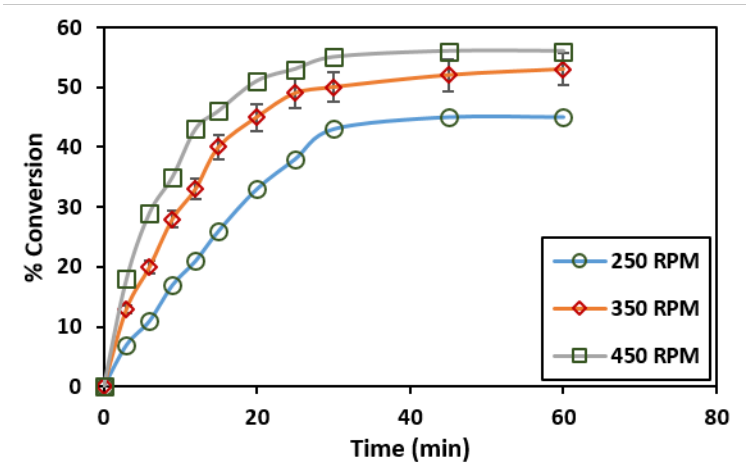
Figure 5.9: Effect of catalyst loading in batch (rotation speed 500 RPM)

mixing and an improved contact between the catalyst and the reactant [9]. This also shows that the mass transfer resistance is overcome in the SMDR as the thin film promotes faster transport of reactant molecules compared to the batch reactor. With an increase in flowrate from 2 ml s^{-1} to 5ml s^{-1}, there was an average increase of 4% in the reaction conversion (Fig 5.10(b)).

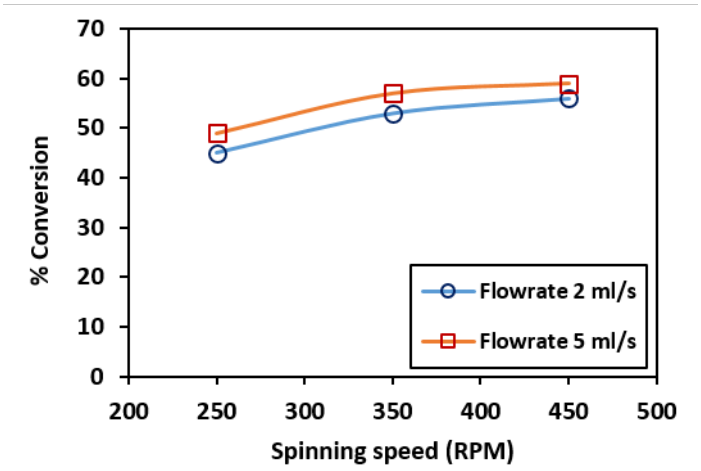
This is due to a greater contact frequency between the catalyst cloth and the reaction solution with an increase in the flowrate. There was a marginal increase in the conversion with flowrate. The initial reaction rate for copper wool in the SMDR was similar to that of free copper in batch (0.0003 mmol s^{-1}) signifying that mass transfer was successfully overcome in the SMDR. Also, the initial reaction rate in the SMDR using copper wool (0.0004 mmol s^{-1}) was found to be twice the rate in batch (0.0002 mmol s^{-1}) under similar operating conditions (Appendix C). Kinetic study showed that the reaction followed 1^{st} order kinetics and the reaction rate constants for the different catalyst system is as seen in Fig 5.10(c). The reaction proceeded at a faster rate in the SMDR using copper wool compared to that in batch and the reaction rate was similar to that obtained using free catalyst in batch. This further indicates the potential of the SMDR for intensification of this reaction. Although the reaction yield is not high as compared to other reports in literature, this study aims to demonstrate the reaction scale-up using a new catalyst system [2, 3].

5.8 Conclusion

In this study, the oxidative homocoupling of 4-methoxybenzylamine was investigated using copper triflate immobilised on wool as a catalyst in batch and the SMDR. A maximum of 70% conversion was observed with the free copper catalyst in batch before product degradation and a conversion



(a)



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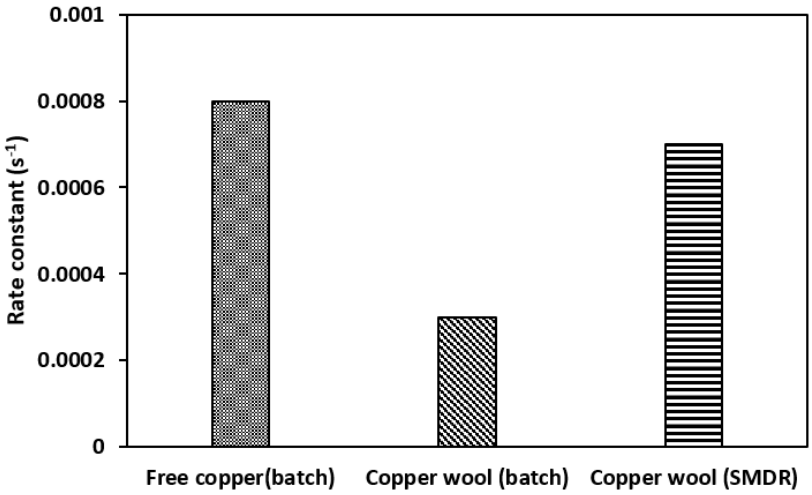

(c)

Figure 5.10: (a) Effect of spinning speed on reaction conversion, (b) Effect of flowrate and (c) Reaction rates for different catalyst systems at spinning speed 450 RPM and 5 ml s^{-1} flowrate.

of 38% was achieve with the immobilised catalyst. However, the yield was 7% higher using the immobilised catalystcompared to the free copeer triflate.The copper cloth was used for the

reaction in the SMDR. An 11% increase was achieved in the reaction conversion with an increase in the spinning speed. Also, the reaction rate in the SMDR using the copper cloth was 2.3 times higher than the reaction rate in batch indicating that mass transfer resistances were overcome in the SMDR. Although the reaction yield was lesser compared to other reports in the literature, the study presents the preliminary results for reaction scale-up using a new catalyst system. Future work should involve a detailed study of the reaction mechanism and further kinetic investigation.

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Chapter 6

Solar Light Photocatalysis in a Rotor-Stator Spinning Disk Reactor (rs-SDR)

The previous chapters have demonstrated the use of a novel reactor design, the SMDR for process intensification. Another route for achieving reaction intensification is by adding multi-functionality to the reactor or by carrying out synergistic reactions. Synergistic processing helps in the reduction of unit operations and downstream processing, hence reducing the amount of waste generated and energy costs [1]. One such example is the application of an SDR for photocatalytic reaction with a UV light source, which has been extensively reported for environmental applications [2]. With recent advances in visible light photocatalysis [3], there is potential to carry out synergistic reactions for fine chemical synthesis.

The rotor-stator spinning disc reactor (rs-SDR) is a modification of the SDR and has shown potential for intensification of multiphase reactions [4]. This study, carried out in collaboration with TU Eindhoven, demonstrates for the first time, visible light photocatalysis in the reactor. The oxidation of methionine with methylene blue was chosen as a photocatalyst to demonstrate the proof of concept. The reactor has been characterized for a range of different parameters and the results have been presented in the following paper.

Initial tests were carried out in batch to determine a suitable light source.The reaction (methodology is detailed in the paper) was first carried out in batch using LED light strips (3.85 mW/nm) as the light source. The effect of catalyst concentration was studied on the reaction rate (Appendix D). The reaction followed pseudo-first order kinetics and the rate decreased with an increase in the catalyst concentration as the solution turned more opaque allowing for lower light penetration. This is due to the insufficient photon flux for higher catalyst concentration which can be overcome by intensifying the light source. The reaction was also carried out in the rs-SDR illuminated with a series of red LED strips from the top (Appendix D). However, conversions <10% were achieved which is mainly due to the limitation of the light source.

Hence, all further experiments were carried out with the reactor placed under a solar light simulator and the findings will be presented in the following paper. Additional plots for this chapter can be found in Appendix D.

<table>
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<tr><td colspan="10">Solar Light Photocatalysis in a Rotor-Stator Spinning Disk Reactor (rs-SDR): Investigating the Reactor Performance for Photocatalytic Oxidation of Methionine</td></tr>
<tr><td colspan="10">Publication status (tick one)</td></tr>
<tr><td>draft manuscript</td><td>✓</td><td>Submitted</td><td></td><td>In review</td><td></td><td>Accepted</td><td></td><td>Published</td><td></td></tr>
<tr><td>Publication details (reference)</td><td colspan="9">Parimala Shivaprasad, Koen Kuijpers, Arnab Chaudhuri, Emma Anna Carolina Emanuelsson, Timothy Noël, John van der Schaaf. To be submitted to Chemical Engineering and Processing: Process Intensification journal</td></tr>
<tr><td>Candidate's contribution to the paper (detailed, and also given as a percentage).</td><td colspan="9">Formulation of ideas (80%): This work was carried out as a part of the Future Research Leaders grant I was awarded by the UofBath. Emma Emanuelsson and I proposed the idea of carrying out solar light catalysis in the rs-SDR as TU Eindhoven had expertise in both areas. The reaction mechanism was suggested by Koen as they had previously optimised it in a microreactor. I contributed ideas for characterising the reactor based on the individual flow regimes.

Design of methodology (75%): I contributed to developing the experimental plan for the detailed characterisation of the reactor after initial screening tests which were proposed by the co-authors.

Experimental work (80%): I carried out bulk of the experimental work which have been presented in this paper including analysis and data processing.

Presentation of data in journal format (85%): I prepared the draft manuscript with contributions by co-authors for schematic diagrams. The co-authors also gave feedback on individual sections.</td></tr>
<tr><td>Statement from Candidate</td><td colspan="9">This paper reports on original research I conducted during the period of my Higher Degree by Research candidature.</td></tr>
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Abstract

In this study, the application of a rotor-stator spinning disc reactor (rs-SDR) has been demonstrated for a gas-liquid, solar light photocatalytic reaction for the first time. The oxidation of methionine was carried out using methylene blue as the photocatalyst in the reactor illuminated by a solar light simulator. The two flow regimes of the reactor, namely, the film and the dispersed sides were characterized individually based on the reaction conversion using a stainless steel disc. The best performance was achieved when the reactor was operated with the illumination of the dispersed side due to better mixing and gas-liquid mass transfer. The use of a transparent PMMA disc showed that both flow regimes contributed to the reaction conversion in difference to a stainless steel disc. Increase in catalyst concentration and reduction in light intensity resulted in lower reaction conversion due to inefficient light penetration. An increase in feed throughput also caused the conversion to drop due to shorter residence times. A maximum conversion of 65% was observed after a residence time of 10 minutes in the dispersed region. Further, the reaction rate was 20 times faster in the rs-SDR compared to the batch reactor showing the potential of the reactor for intensification of multi-phase photocatalytic reaction.

6.1 Introduction

Photocatalytic chemical and biochemical synthesis is a sustainable alternative to conventional processing as they improve the product selectivity and reduce the energy consumption [5, 6]. Both artificial and solar light induced catalytic reactions have been reported for a wide range of metal and metal free catalysts at a laboratory scale [2, 7, 8]. The lack of industrial application for this technology is related to photon transfer and transport limitations of the conventional reactors used for these reaction systems [9]. Hence, there is a need for a reactor system which can integrate maximum photonic efficiency and mass transfer within a single equipment.

In recent years, process intensification (PI) has been a promising pathway to achieve sustainable chemical production [10]. Process intensified reactors have recently been employed in the form of microreactors, which can overcome light penetration limitations for photocatalytic reactions [8, 9]. However, it still remains challenging to achieve industrial scale photocatalytic reactions with this technique. Adding multi-functionality in PI reactors through a combination of different energy forms is an increasingly popular route for reaction intensification [1]. One such example is the application of the spinning disc reactor (SDR) for photocatalytic advanced oxidation reaction where the combination of gravitational force from the spinning disc and energy from the light source has shown improved reaction efficiency [11–13]. In another study [14], solar light driven oxidation of primary benzylamine catalysed by Eosin Y has been reported in the SDR. The reaction rate in the SDR was nearly 50% lesser compared to batch indicating that the photon efficiency was not completely utilized due to a lower film thickness resulting in irradiation of lower liquid volume. Also, the photocatalyst was dispersed in the reaction solution which led to only a small fraction of catalyst being irradiated within the thin film. Hence, there is scope to further improve the reactor design for efficient photon transfer in rotating disc reactors.

Another modification of the SDR is the the rotor-stator spinning disc reactor, which consists of a rotating disc enclosed by a stator. Reaction intensification is caused by the high shear force on the feed as a result of the velocity gradient between the rotor and the stator; resulting in high rates of mass and heat transfer [4]. The reactor has shown to overcome mass transfer limitations for a range of multi-phase reactions. For gas-liquid reactions, co-feeding of the two phases results in the formation of a thin liquid film (well mixed region) and a dispersed, bubbly region (plug flow). It has been shown that the contribution of the gas-liquid dispersed region has a higher contribution towards the overall mass transfer co-efficient compared to the film region at higher gas flowrates and disc rotation speed [15]. However, the combined configuration has shown to achieve greater gas-liquid mass transfer compared to the conventional SDR, which operates mainly with a thin film regime [15]. This leads to the rs-SDR being a particularly interesting reactor for photochemical applications where photon transfer and mass transfer of the activated species are often limited

(e.g. for high concentration).

Methionine sulfoxide is an important intermediate for the pharmaceutical, material science and organic synthesis applications [16]. Synthesis of methionine sulfoxide proceeds through the oxidation of methionine using singlet oxygen is a well reported mechanism as it is a simple yet an effective route for synthesis [16]. Conventional methods reported for methionine oxidation are accompanied by harsh reaction conditions with a potential for over oxidation of sulfoxides to sulfones resulting in reduced reaction selectivity [17, 18]. Production of singlet oxygen using a photocatalyst is a popular photoxidation route [16]. Dyes have recently shown the potential for visible light driven photoxidation reactions, allowing for a metal-free and non-toxic synthesis pathway [3]. Methylene blue is one such photosensitive dye which has the potential for clean and economic photoxidation of methionine to methionine sulfoxide. Methylene blue immobilised on glass beads has been used for oxidation of methionine with a tungsten lamp as a light source [19]. Although oxidation was successful, it was accompanied by long reaction time and low product yield. The reaction was also carried out only at a scale of 10 ml.

The aim of the present study is thus to demonstrate the potential of the rs-SDR for visible light driven photoxidation of methionine using methylene blue as the catalyst. This is a challenging reaction as it is limited both by gas-liquid mass transfer and photon efficiency, and it is the first time such a reaction system is being investigated in an intensified reactor. The reactor performance will be characterised based on spinning speed, feed flowrate, catalyst concentration, disc material and light intensity for the two flow regimes individually.

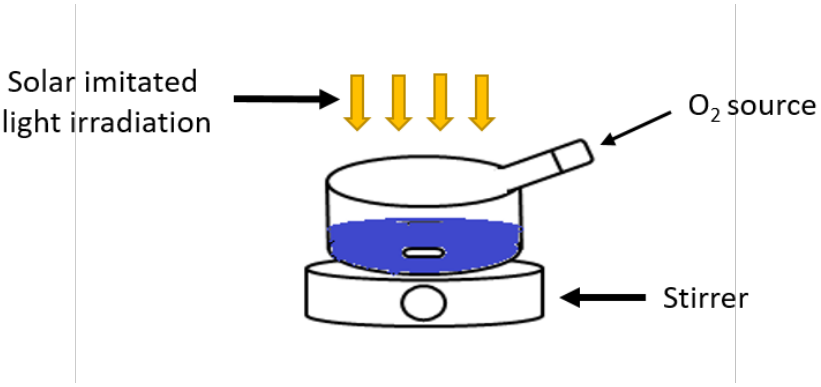
6.2 Materials and methods

6.2.1 *Materials*

Methionine was obtained from Fluorochem and methylene blue was procured form Merck and used as obtained.

6.2.2 *Reaction in batch*

The experimental setup for the batch reaction is as shown in Fig 6.1. A sealed glass trough with a side stopper was used for the reaction. The typical reaction scheme is as represented in Fig 6.2. The reaction mixture containing 0.1 M methionine and 1 mM methylene blue catalyst in 15 ml deionized water was first saturated with a constant flow of oxygen for 3 hours. The reaction solution was then introduced into the glass trough placed directly beneath the solar light simulator. The distance between the light source and the reaction vessel was maintained to ensure maximum irradiation intensity. The space around the reaction vessel was air cooled to ensure minimum



(a)

Figure 6.1: Experimental set-up for batch reaction

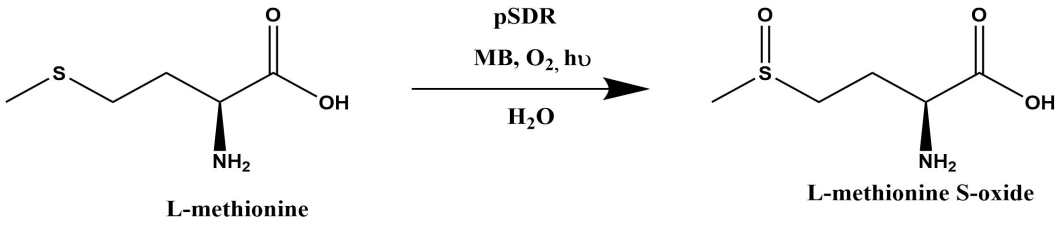


Figure 6.2: Reaction scheme for photoxidation of L-methionine with the end product L-methionine sulfoxide

temperature variation. The reaction was carried out for 90 minutes and 0.1 ml of aliquots were collected at 10, 20, 30, 60 and 90 minutes. The samples were diluted and measured using a Shimadzu HPLC (UV detector) fitted with a Grace Smart RP18 column using 95% water+0.1% TFA and 5% acetonitirile as the mobile phase. The reaction conversion was calculated from the peak areas of the substrate and the product.

Methionine oxidation has shown to follow pseudo-first order reaction kinetics as follows [16]:

$$r = -\frac{d[Met]}{dt} = k_{app}.[Met] \tag{6.1}$$

The integration of the above equation between the initial and final methionine concentration results in the following equation:

$$-ln\frac{[Met]}{[Met_0]} = k_{app}.t \tag{6.2}$$

Where, r is the reaction rate (mol L^{-1} s^{-1}) and k_{app} is the apparent first order constant (s^{-1}) and t is the reaction time (s).

The initial reaction rate was calculated by multiplying k_{app} (obtained from the slope of the above curve fit) by the initial methionine concentration.

6.2.3 *Reaction in the photo rs-SDR*

The detailed configuration of a co-fed rs-SDR is published elsewhere [15]. The reactor was illuminated using a solar light simulator (AM1.5G),with a 1200W halogen light source. Both top and bottom co-feed configurations were investigated as the reactor has two flow regimes (Fig 6.3 (a) and (b)), the film and the dispersed region depending on the position of the feed inlet. The film and dispersed regions in the reactor have different liquid volumes and flow characteristics which in turn has an effect on the light penetration and mass transfer. Hence, in each configuration either the film or the dispersed regime were closest to the light source. Further, two different disc/rotor materials were used, polymethyl methacrylate (PMMA) of radius 130 mm and stainless steel (SS) of radius 132 mm. All reaction characterisation experiments have been carried out using SS disc to allow for a comparison between the film and dispersed regimes.

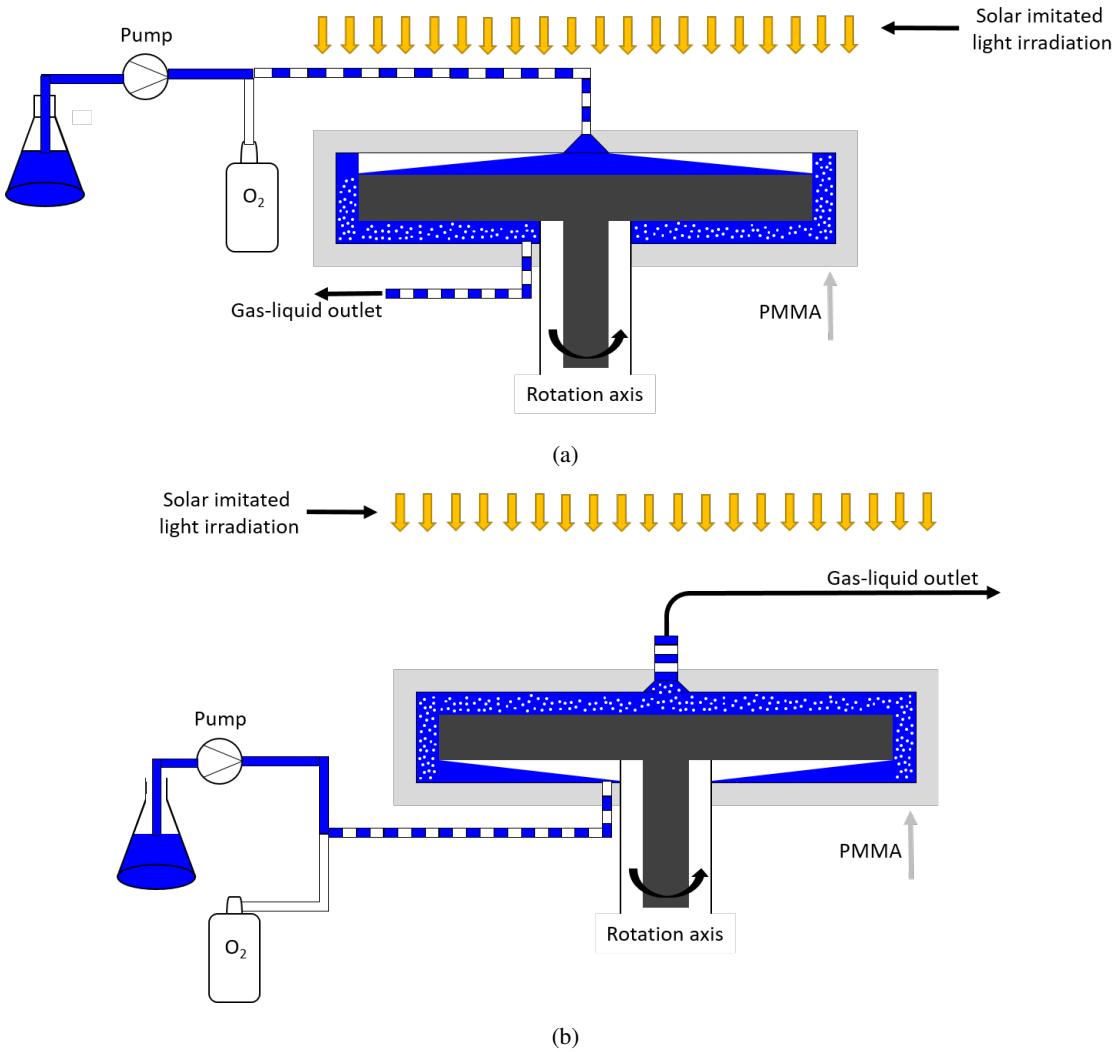


Figure 6.3: (a) The rs-SDR configuration based on the feed position: (a) top co-fed and (b) bottom co-fed

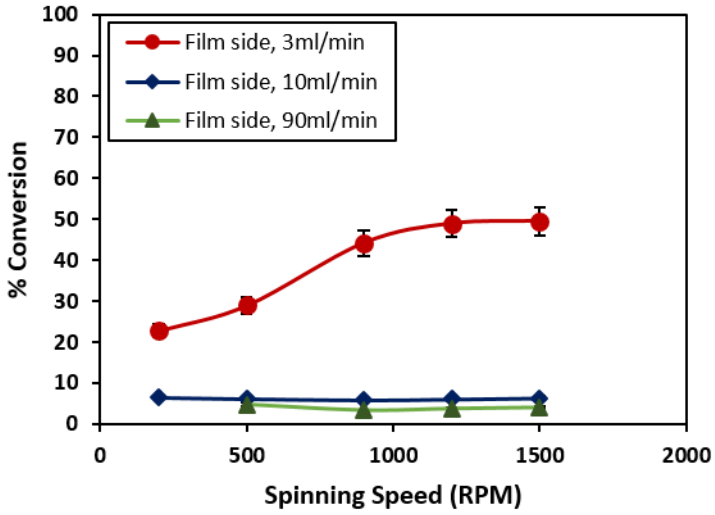
The reaction solution was prepared by dissolving L-methionine (0.1M) and methylene blue (desired concentration) in the 1 L deionised water. The reaction was carried out by co-feeding oxygen from a Bronkhorst mass flow controller and the reaction solution either from the top or the bottom of the reactor for different rotational speeds. The liquid:gas flowrate was maintained at a stoichiometric ratio of 1:2.5. Samples were collected after a single pass through the reactor and the reaction conversion was measured using HPLC similar to the method outlined in the previous section.

6.3 Results and discussion

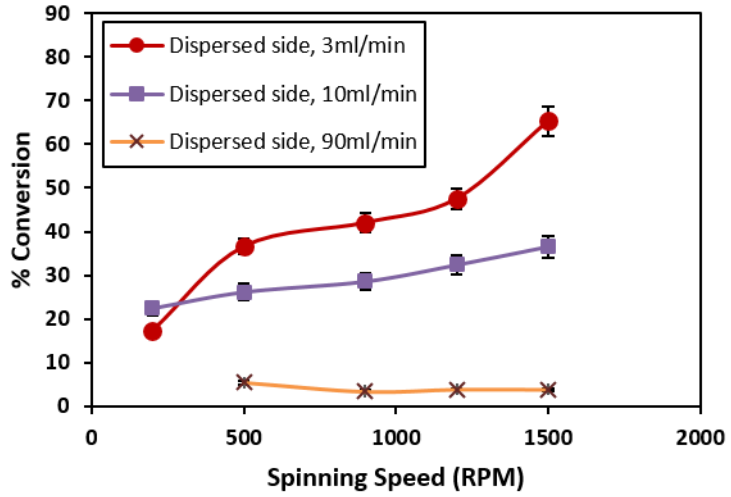
6.3.1 *Region of illumination*

The contribution of the film and the dispersed side to the reaction was quantified using a stainless steel disc to ensure no light penetration into the opposite region of illumination. Fig 6.4(a) shows the film side contribution with a maximum conversion of 50% achieved with a liquid flowrate of 3 ml min^{-1}. The reaction conversion decreased thereafter for higher liquid flowrates. This may be due to: (i) a lower residence time, despite the larger film thickness due to the higher flowrate (Appendix D) and/or (ii) inefficient gas-liquid mixing within the thin film resulting in kinetic limitation. This is contrary to the observations made in a conventional SDR for a single phase photocatalytic reaction, where 'flooding' conditions have shown to have improved the reaction rate compared to that of a thin film [14].

Fig 6.4(b) shows the reaction conversion from the dispersed side with a maximum conversion of 65% for 3 ml min^{-1}. For liquid flowrate of 10 ml min^{-1}, the conversion was nearly 30% higher than the film side conversion. Previous report has shown that the volumetric gas-liquid mass transfer is 4 to 6 times higher compared to the film side with increasing rotation speeds, allowing for better mixing between the two phases [15]. Visual studies (Appendix D) show the formation of large gas bubbles at lower rotational speed accompanied by lower gas-liquid mass transfer. With an increase in spinning speed, the frequency of formation of the large gas bubbles is lower compared to the number of smaller gas-bubbles which shear off from the rim of the rotor. This increases the turbulence in the dispersed region accompanied by a higher mass transfer and hence a higher reaction conversion is observed at higher disc rotation speeds for both feed throughput. When the flowrate was increased to 90 ml min^{-1}, it resulted in <5% reaction conversion in both film and dispersed regions. An increase in liquid flowrate results in a shorter residence time (Appendix D) and an increase in the volume of liquid in the region between the rotor and the top stator. This causes a reduction in light penetration and illumination time for both flow regimes resulting in lower conversions.



(a)



(b)

Figure 6.4: Contribution to reaction conversion by: (a) film side and (b) dispersed side. The reaction was carried out with 1mM catalyst and maximum light intensity.

The comparison between the initial reaction rate in batch and the dispersed side of rs-SDR is as shown in Fig 6.5. The reaction in the rs-SDR was found to be 7 times faster compared to the reaction in batch reactor indicating better light penetration and mass transfer was achieved in the reactor.

6.3.2 *Material of the disc*

The reactions were carried out with PMMA and SS discs to investigate if both flow regimes contributed to the reaction when operated with PMMA disc. Fig 6.6(a) shows the reaction conversion was higher with PMMA disc and a maximum conversion of 60% was observed at liquid flowrate

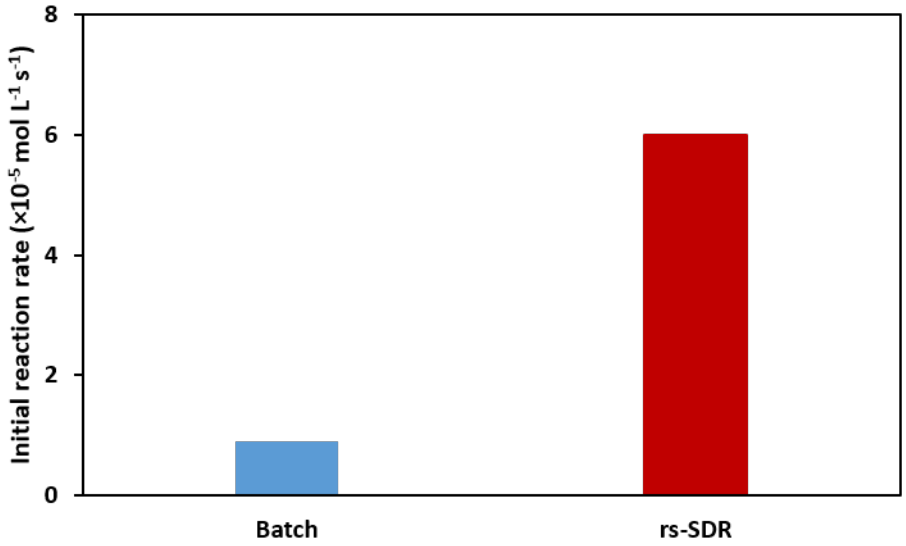
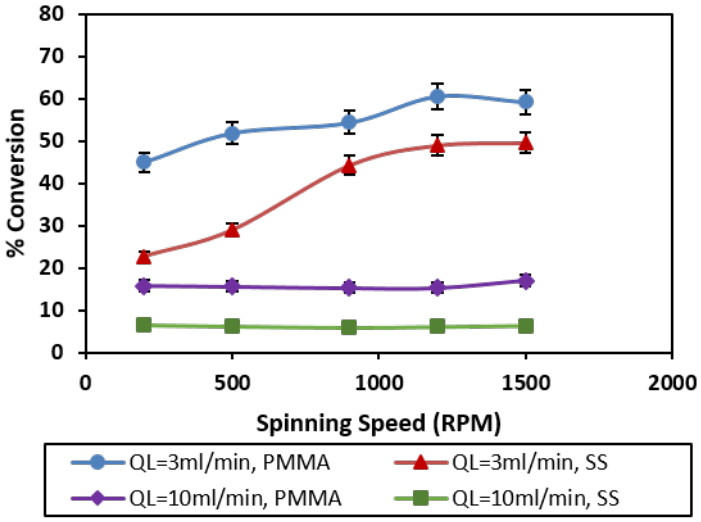


Figure 6.5: Initial reaction rate comparison between batch and rs-SDR

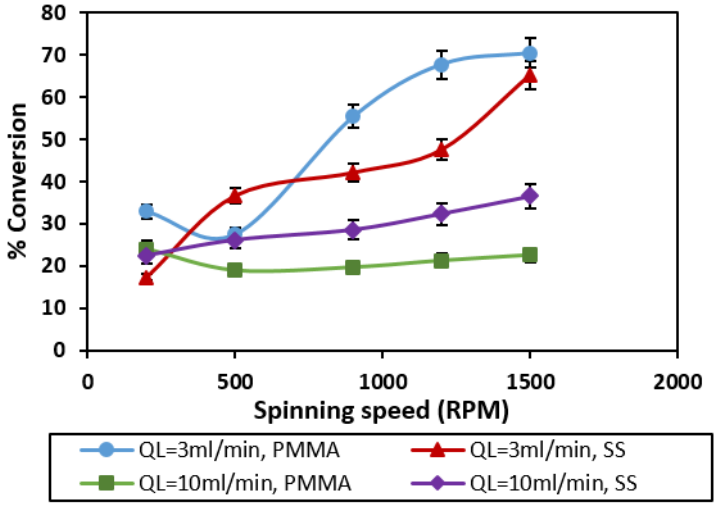
of 3 ml min^{-1}. The liquid film has a high surface shear and hence a lower liquid volume compared to the dispersed region improving light penetration and mass transfer within the thin film. Further, the PMMA disc is transparent and some photons may also penetrate through the disc and into the dispersed region resulting in both regions contributing for the reaction conversion. A similar trend in the dispersed region (Fig 6.6(b)) was observed for flowrate 3 ml min^{-1}. Even though liquid volume is higher in the dispersed region, there may still be some light penetration into the dispersed side through the film side at this flowrate. However, the reaction conversion with SS disc was found to be 10% higher than the PMMA disc for flowrate of 10 ml min^{-1}. This suggests that the SS disc may be reflecting the photons back into the reaction solution increasing the photon flux compared to the PMMA disc.

6.3.3 *Catalyst concentration*

The catalyst concentration is an important parameter in photocatalytic reactions as the opacity of the solution influences light penetration. Methylene blue concentration of 1 mM and 10 mM was chosen to investigate the effect on reaction conversion. The reactions were carried out using SS disc. In the film side (Fig 6.7(a)), the conversion decreased by 28% with increase in catalyst concentration for 3 ml min^{-1} flowrate. At this flowrate, spiral like patterns were observed at lower spinning speeds instead of a thin film (Appendix D). This may have affected the light penetration with increase in catalyst concentration as the reaction solution was mostly opaque. However, the difference in reaction conversion for flowrate of 10 ml min^{-1} was <5%. A more uniform film region was observed at this flowrate resulting in a thin film region of lower opacity and hence allowing for better light penetration for both catalyst concentrations. In the dispersed side (Fig



(a)



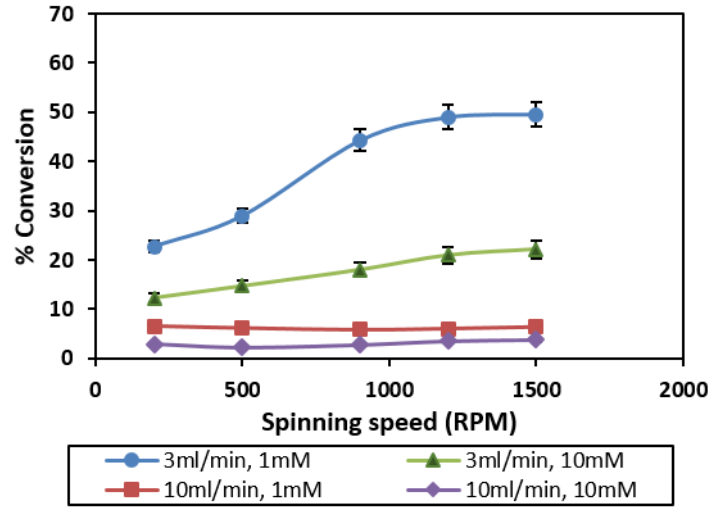
(b)

Figure 6.6: Effect of disc material on reaction conversion: (a) film side and (b) dispersed side. The reaction was carried out with 1mM catalyst and maximum light intensity.

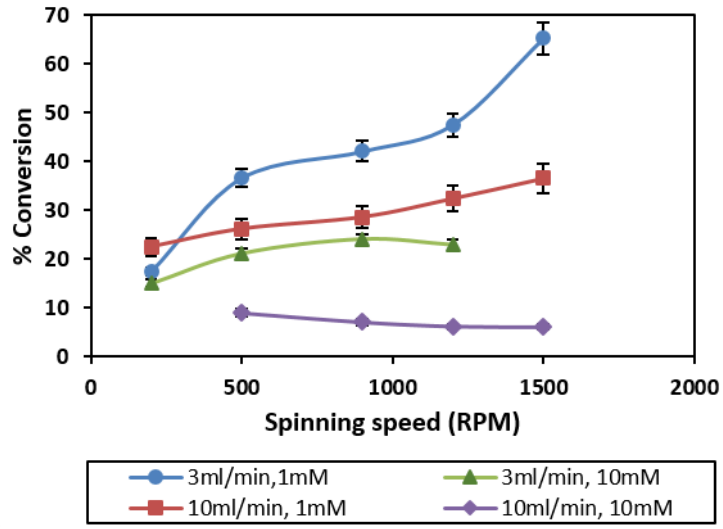
6.7(b)), there was a significant drop in conversion with increase in catalyst concentration for both flowrates. Theoretical light transmittance of methylene blue (Appendix D) indicates a drastic drop in transmittance with increasing liquid depth and catalyst concentration, reducing light penetration in the dispersed region.

6.3.4 *Light intensity*

The effect of light intensity was measured by adjusting the lamp intensity between the minimum and maximum setting, while a constant distance between the lamp and top of the reactor was maintained. The reactions were carried out using SS disc. As expected, the reaction conversion decreased with the minimum light intensity in both film and dispersed regions. As seen in Fig



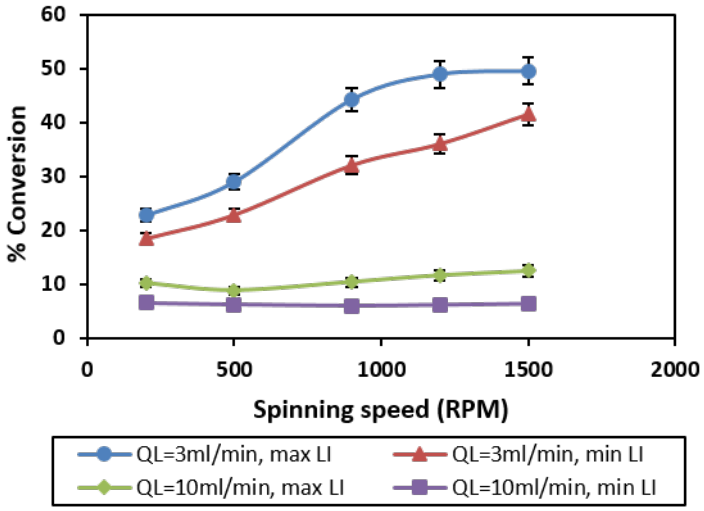
(a)



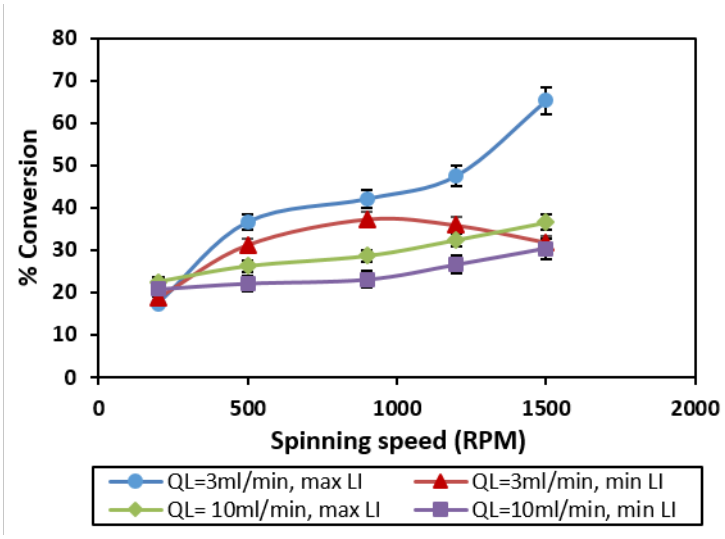
(b)

Figure 6.7: Effect of catalyst concentration on reaction conversion: (a) film side and (b) dispersed side. The reaction was carried out disc at maximum light intensity

6.8(a), the difference in reaction conversion between minimum and maximum light intensity was on an average 8% and 6% from 3 ml min^{-1} to 10 ml min^{-1} liquid flowrate. The film thickness also decreases with increasing spinning speed allowing for better light penetration even for a lower photon flux. Hence, the film layer is uniformly illuminated at both light intensities. A similar trend was observed in the dispersed region (Fig 6.8(b)). This is an interesting observation as the dispersed side holds the maximum liquid volume and a greater drop in reaction conversion is expected with a lower light intensity as the mixture is not expected to be irradiated homogeneously. For a given catalyst concentration, the maximum efficiency of the catalyst may not vary with change in light intensity and the same was observed in the dispersed side.



(a)



(b)

Figure 6.8: Effect of light intensity in: (a) film side and (b) dispersed side. Catalyst concentration of 1mM was used for the reaction.

6.4 Conclusion

In this study, the application of the rs-SDR has been investigated for a gas-liquid photocatalytic reaction for the first time. Solar light driven, oxidation of methionine was carried out using methylene blue as the photocatalyst. The reaction conversion in the film and dispersed flow regimes were individually evaluated based on the region closest to the source of illumination. The overall reaction conversion in the dispersed region for a lower feed throughput was higher compared to the film region as it has a greater contribution to the gas-liquid mass transfer. PMMA disc allowed for light penetration into the dispersed region when operated in the film regime, increasing the reaction conversion compared to the SS disc. A ten-fold increase in catalyst concentration caused a drastic drop in conversion in film side for lower feed throughput due to the incomplete forma-

tion of the thin film. The significant drop in conversion was also observed in the dispersed region due to insufficient light penetration with increasing opacity of the reaction liquid. The reaction conversion was also found to be influenced by the light intensity in both regimes. A maximum reaction conversion of 65% was achieved for the liquid flowrate of 3 ml min^{-1} and a catalyst loading of 1 mM with a residence time of 10 minutes. Although the conversion dropped by 30% for higher feed flowrate, this was mainly due to photon efficiency as not all incident photons were absorbed by the reaction system.Overall, the results indicate that the rs-SDR is best operated with the dispersed region closest to the illumination source. Further,the reaction rate was found to be 20 times faster in the rs-SDR compared to batch indicating the feasibility of the reactor for multi-phase photocatalytic reactions. Hence, the combination of the two flow regimes characteristic of the rs-SDR is key for achieving gas-liquid phase photochemical reaction which is not present in a conventional SDR. Hence, this study successfully demonstrates the proof of concept for the application of rs-SDR for a two-phase synergistic reaction with scope for improving photon efficiency to facilitate reactions with higher feed throughputs in the future. Additionally, contact angle and light intensity measurements will have to carrried out to estimate the wettability of the disc surface and the amount of light reaching the two flow regimes within the reactor.

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Chapter 7

Conclusion and recommendations

The overall conclusions from the preceding chapters and recommendations for future work are presented in this chapter.

7.1 Conclusion

To conclude, the application of the spinning mesh disc reactor (SMDR) has been investigated for intensification of new and challenging chemistry. The SMDR has shown the potential to scale-up and increase the productivity of organic reactions catalysed by both chemo and enzyme catalysts compared to conventional batch reactor. A different route to scale-up in the reactor was also demonstrated by increasing the cloth size and the cloth number and hence the catalyst loading. Another modification of the SDR, the rs-SDR was used for photocatalytic reaction demonstrating the proof of concept for synergistic reactions in a PI reactor. The main findings from the present work are as follows:

1. Intensification of Henry reaction in the SMDR was investigated using copper triflate immobilised on wool. First, a simple protocol was developed for the immobilisation of copper triflate on wool and the catalyst cloth was fully characterised using techniques like SEM, EDX and XPS to confirm the presence of copper on wool. Henry reaction was carried out in batch using both free and immobilised catalyst and a reaction conversion of 57% and 85% was obtained respectively after 24 hours. The formation of a copper-protein complex upon immobilisation, proved to be a better catalyst compared to free copper triflate. The reaction was then carried out in the SMDR with the copper cloth as the catalyst. The effect of spinning speed and flowrate on reaction conversion was measured and a maximum conversion of 90% was achieved at the spinning speed of 350 RPM and a flowrate of 5 ml s^{-1} after 5 hours. Reaction intensification in the SMDR was due to an increased average surface shear resulting in better mixing and lower mass transfer resistance. Wool in its natural form also showed catalytic properties for the reaction but showed poor reusability for multiple cycles compared to copper wool.

2. A range of new process intensified reactors for enzyme catalysed organic synthesis have been reviewed and opportunities for translating this technology to the industry has been discussed.

3. The application of the SMDR was extended for enzymatic resolution. Kinetic resolution of 1-phenylethanol was investigated using an inexpensive amano lipase. Lipase from *pseudomonas fluorescens* was immobilised on wool and the lipase cloth was characterised to verify immobilisation. The kinetic resolution reaction was first carried out in batch to identify the best solvent and operating temperature for both free and immobilised lipase. Maximum reaction conversion was observed at 25°C using ethyl acetate as the solvent. The reaction was then carried out in the SMDR using both free and immobilised lipase and the reactor parameters were optimised. A productivity of 11 g l^{-1} h^{-1} was achieved in the SMDR using the lipase cloth, which was 30% higher compared to the productivity in batch reactor. Reaction scale-up in the SMDR was achieved for higher feed throughputs without a loss in the reaction efficiency compared to other reports for the same reaction system in literature. The cost of *pseudomonas fluorescens* per 10g is twice lower than that of non-amano lipase immobilised on commercial supports. Hence, the choice of enzyme was also dependent on reducing the overall cost of the process. Further, the catalyst loading was increased by adding more lipase cloths and the reaction rate doubled from one to three cloths. The lipase cloth maintained an activity of 83% upon being re-used for three cycles. The kinetics of the reaction conformed well to Ping Pong bi-bi mechanism. A one-pot chemo-enzymatic cascade reaction was attempted using the above reaction system. However, the reaction was found to be limited by catalyst deactivation in the reaction medium in one-pot.

4. A newly designed SMDR was used to investigate two different routes for reactor scale-up, namely, increase in cloth size and cloth number. Hydrolysis of tributyrin and Henry reaction were chosen as they had previously been optimised in the reactor. A full factorial DoE study was first used to investigate the effect of spinning speed, cloth size and cloth number on the reaction rate. An increase in cloth size resulted in an increase in reaction rate due to the increase in the cloth surface area and hence the amount of catalyst immobilised. Addition of cloths of increasing cloth size also improved the reaction rate at higher substrate concentrations. For tributyrin hydrolysis, maximum reaction rate of 6.9 mM min^{-1} was obtained for three 50 cm cloths at 99mM substrate concentration. Similarly, for Henry reaction, the reaction rate increased with the increase in cloth size and cloth number with a maximum reaction rate of 0.043 mmol min^{-1} for three 50 cm cloths.

5. The application of the reactor was also extended for imine synthesis via oxidative homocoupling of 4-methoxybenzylamine. Copper triflate was used as a catalyst both in its free form and immobilised on wool. The copper cloth proved to be a better catalyst as the reaction yield was 7% higher compared to the free catalyst in batch. The reaction using free catalyst resulted in product degradation over a prolonged reaction time. In the SMDR, the reaction conversion increased with increase in spinning speed and flowrate and a maximum conversion of 56% was

achieved at 450 RPM and a flowrate of 5 ml s^{-1}. The reaction rate in the SMDR using the copper cloth was found to be 2.3 times higher than the batch reactor indicating mass transfer resistance was overcome in the SMDR.

6. The potential of the rs-SDR for visible light photocatalytic reaction was demonstrated for the oxidation of methionine using methylene blue as the photocatalyst. The film and dispersed regions of the reactor were individually characterised for various reactor parameters. The overall conversion in the dispersed region was found to be higher than the film side as the gas-liquid mass transfer is higher. In comparison to batch, the reaction was 20 times faster in the dispersed side of the rs-SDR. When the reactor was operated in the film regime and a PMMA disc, it was found that both flow regimes contributed for the reaction as the photons were able to pass through the thin film and into the dispersed region. An increase in catalyst concentration resulted in a lower reaction conversion due to light limitation with increasing opaqueness of the reaction solution. Similarly, a lower light intensity resulted in lower conversion.

7.2 Recommendations

The following recommendations can be implemented to further extend the work presented in this thesis.

1. Till date only woollen cloth has been used as a catalyst support in the reactor and there is scope to test other support materials. Although catalyst immobilisation on carbon cloth and glass fibre was unsuccessful in the present study, it was mainly due to the harsh pre-treatment conditions. Less invasive pre-treatment techniques like plasma treatment can be explored to introduce functional groups. Other supports like zeolite foams and MOFs can also be potential supports as the desired catalyst can be incorporated during the fabrication of support. Process intensification till date has been achieved either by using a novel technique or a procesing method and the way forward is to novel materials (as catalysts or supports) to achieve intensification of catalytic reactions is the way forward.

2. The protocol developed for copper triflate immobilisation on wool can be extended to other metal and metal organic catalysts which will allow for a wider range of reactions in the SMDR. Similarly, it can also be used for immobilising different strains of lipase to further improve the selectivity and yield of enzymatic reactions in the SMDR. Also, the copper-wool catalyst showed interesting catalytic properties (as seen in imine synthesis) due to the complex formation with the amino acid groups present in wool. This should be further investigated to better understand the effect of natural catalyst supports on the reaction mechanism.

3. One of the challenges with the one-pot reaction in the present study was the incompatibility of

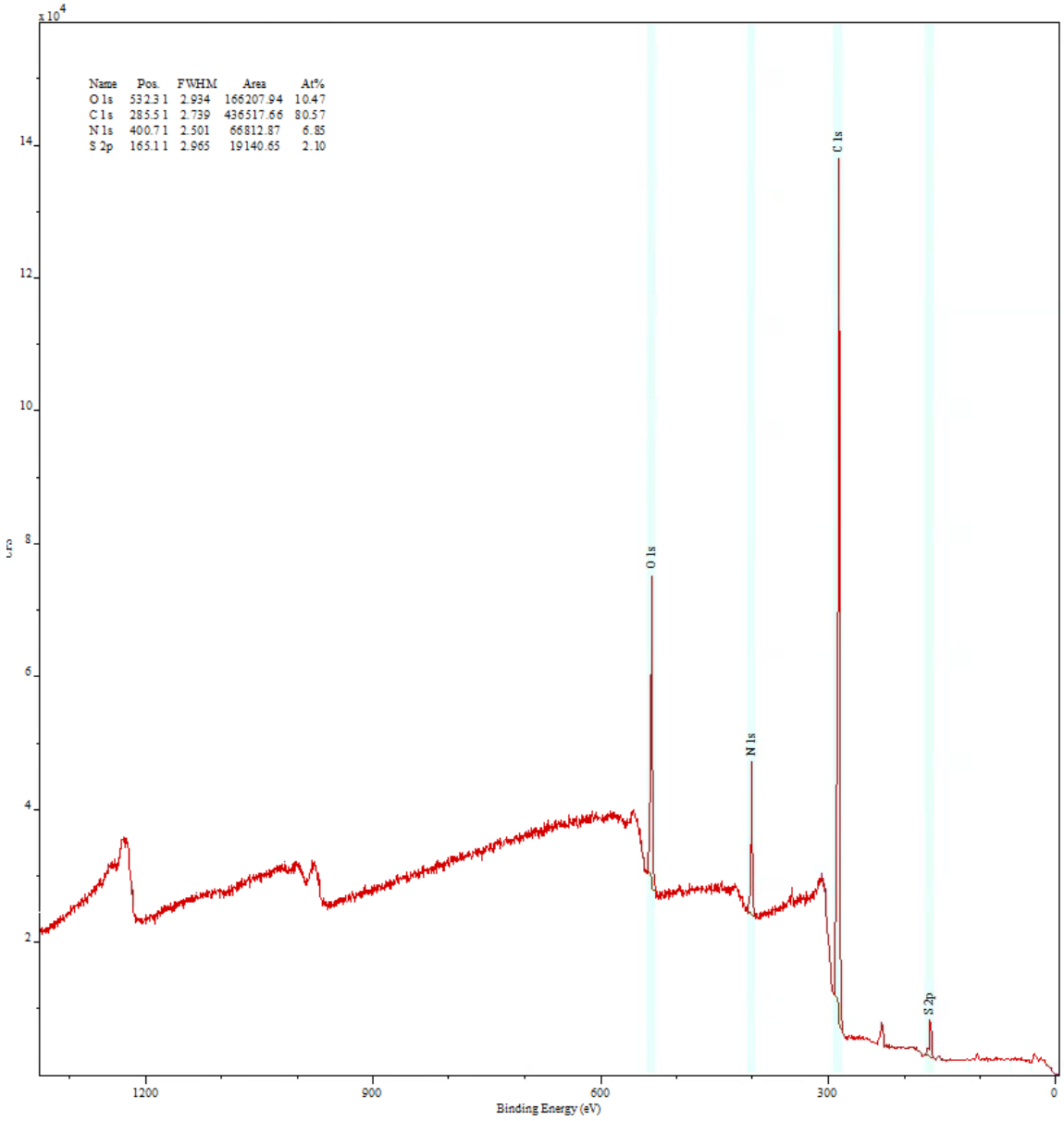
wool with the best performing solvent for the reaction. This can be overcome by using inert supports like carbon cloth and foams, which offer minimum interaction with the solvent and the reaction. It is also important to test different combinations of the chemical and the biological catalyst in one pot for chemo-enzymatic cascade reactions to ensure catalyst deactivation doesn't occur for a given reaction medium. Another way to improve the potential to achieve a one-pot reaction in the SMDR would be to separate the feeding ports for the two catalyst cloth discs to prevent catalyst poisoning due to the reagents present in the reaction mixture.

4. The study showed the potential of the SMDR for a range of organic reactions with optimisation and scale-up studies. However, to enable application at a pilot scale, an in-depth economic and life cycle analysis has to be carried out. This will provide insight into the feasibility of using the SMDR against existing reactors in small and medium scale chemical industries. Further, the energy consumption of the SMDR has to be benchamrked against a conventional stirred tank reactor to enable a fair economic analysis.

5. The proof of concept was demonstrated for visible light catalytic reaction in the rs-SDR. There is further scope to intensify the light source to minimise light limitation at higher catalyst concentrations. Also, the gas flowrate and rotation speed can be further increased to improve the gas-liquid mass transfer. The reactor used in the present study could only operate for aqueous phase reactions as the reactor was made of PMMA. A new reactor with suitable material of construction has now been commissioned and will be tested in the future for a wider range of reactions with specific application to the pharmaceutical industries.

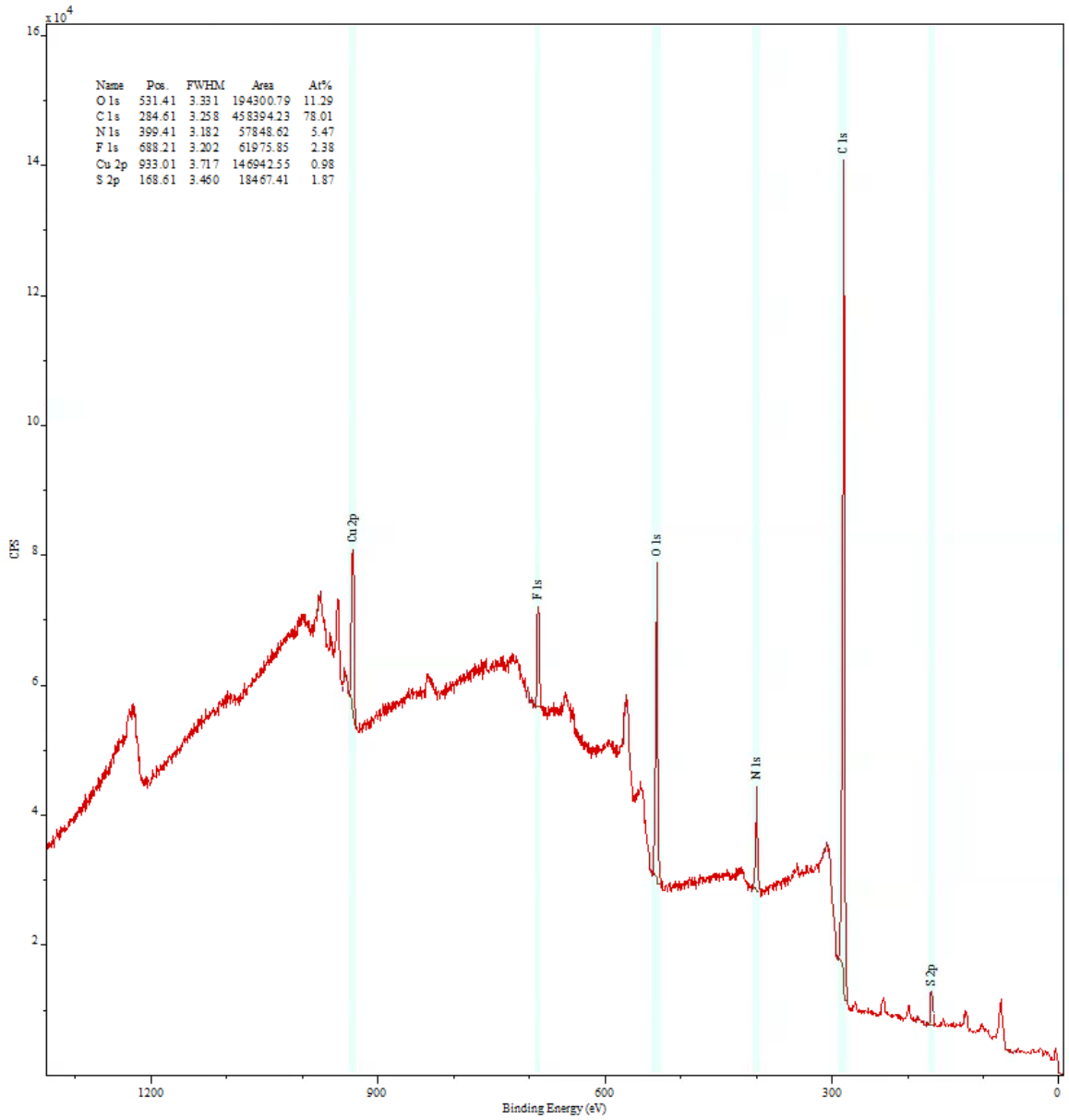
Appendix A

Supplementary information for Chapter 2



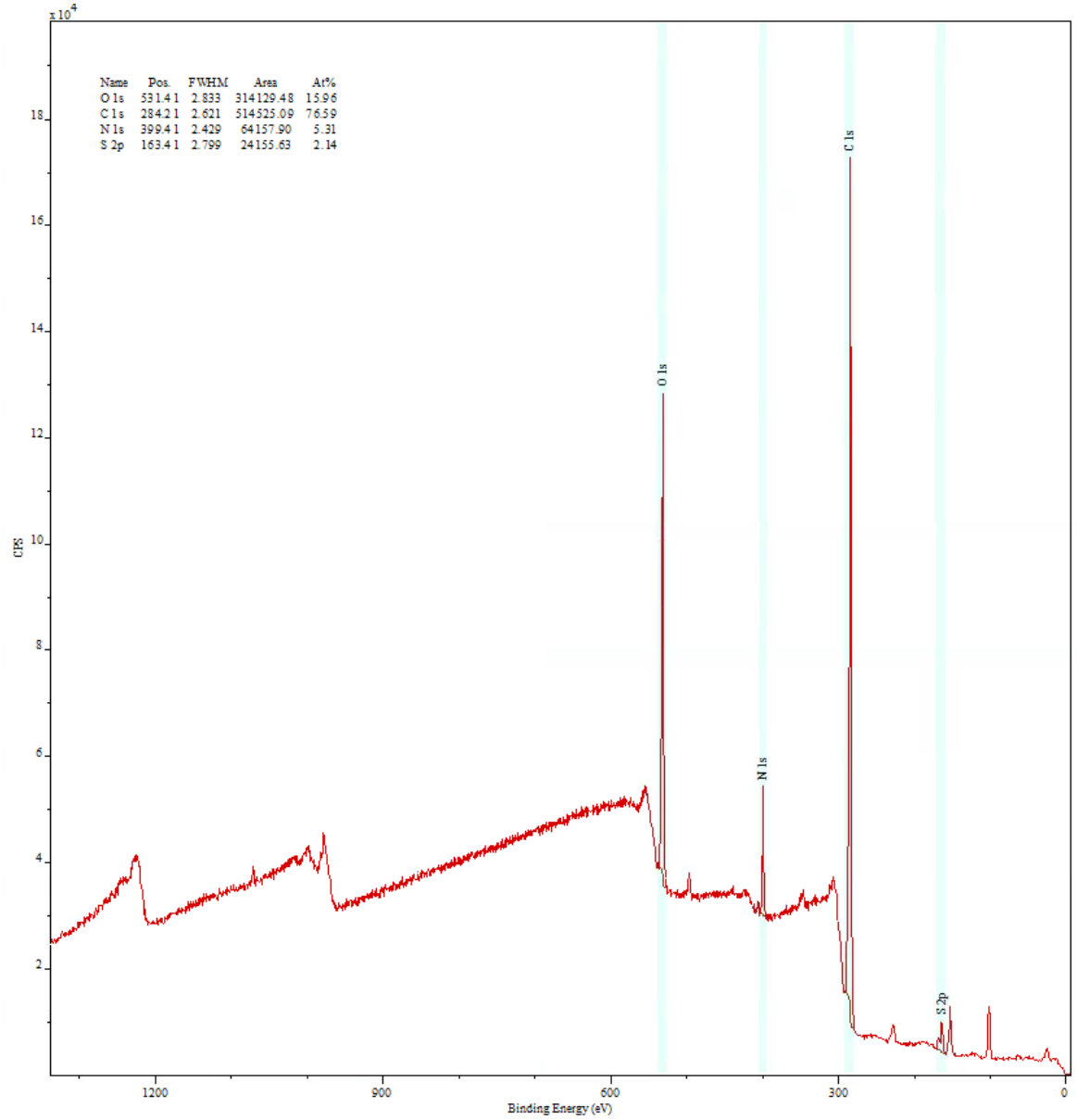
(a)

Figure A.1: Survey spectrum of plain wool from XPS



(a)

Figure A.2: Survey spectrum of copper woo from XPSl



(a)

Figure A.3: Survey spectrum of re-used plain wool from XPS

Additional figures for chapter 2

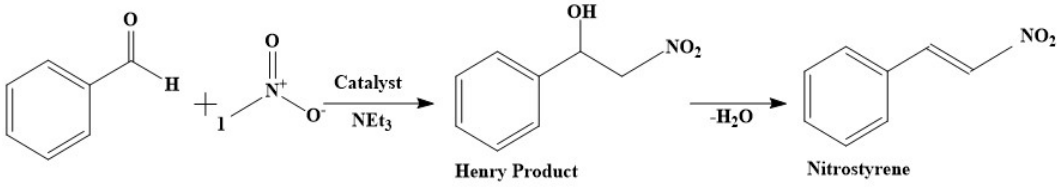
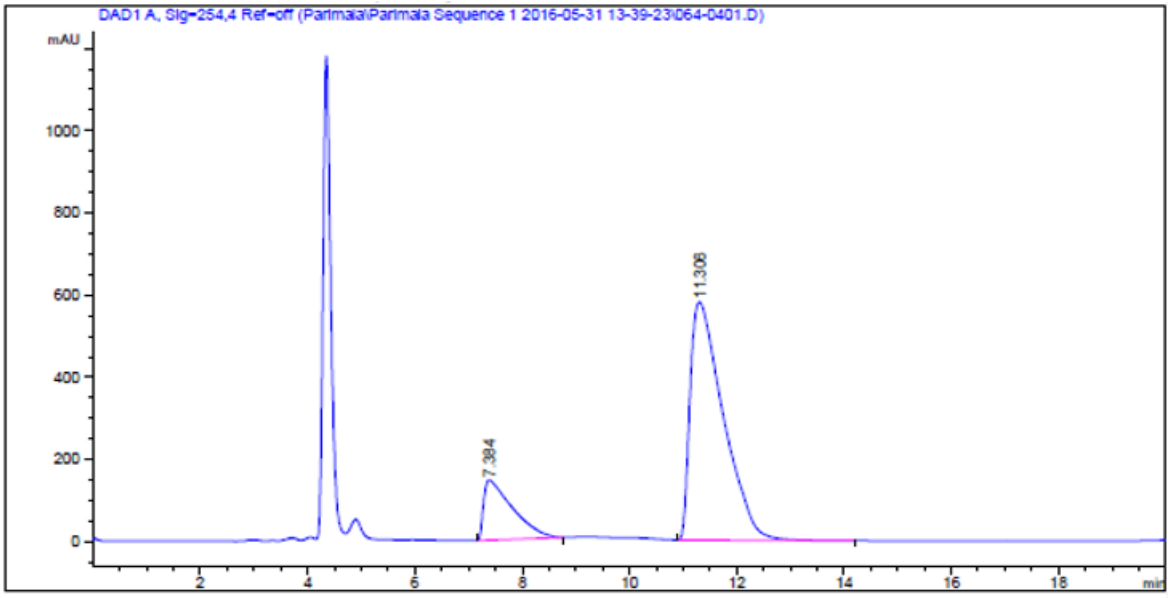


Figure A.4: Reaction scheme for nitroaldol condenstation reaction



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.933	MM	0.6244	2252.89819	60.13093	13.6554
2	11.796	MM	0.7162	1.42453e4	331.50119	86.3446

Figure A.5: HPLC spectrum indicating the two enantiomers of the product from nitroaldol condensation reaction. The R enantiomer has a retention time of 7.93 min and the S enantiomer has a retention time of 11.76 minutes.

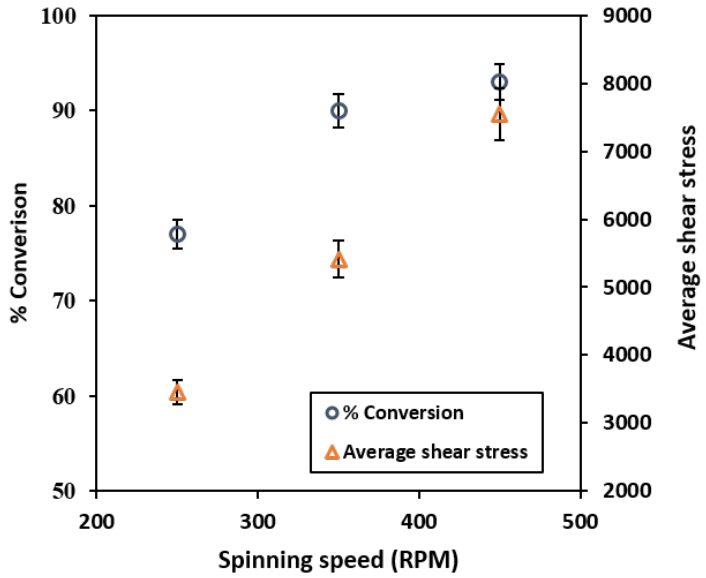


Figure A.6: Improved plot for conversion vs shear stress

Appendix B

Supplementary information for Chapter 4

FTIR of plain and lipase wool

Natural wool is characterised by a broad vibration at 3280 cm^{-1} due to N-H stretches. The vibrations at 1633 cm^{-1}, 1525 cm^{-1} and 1236 cm^{-1} are due to the S-H and N-H functional groups found in wool. The new vibration at 3070 cm^{-1} and 1392 cm^{-1} can be attributed to the N-H stretching mode due to the presence of PEI after the surface modification step. Along with the reduced intensity of the vibration at 2929 cm^{-1}, it can be inferred that PEI was successfully immobilised on wool. The immobilisation of lipase can be implied by the reduced intensity of the 2929 cm^{-1} vibration.

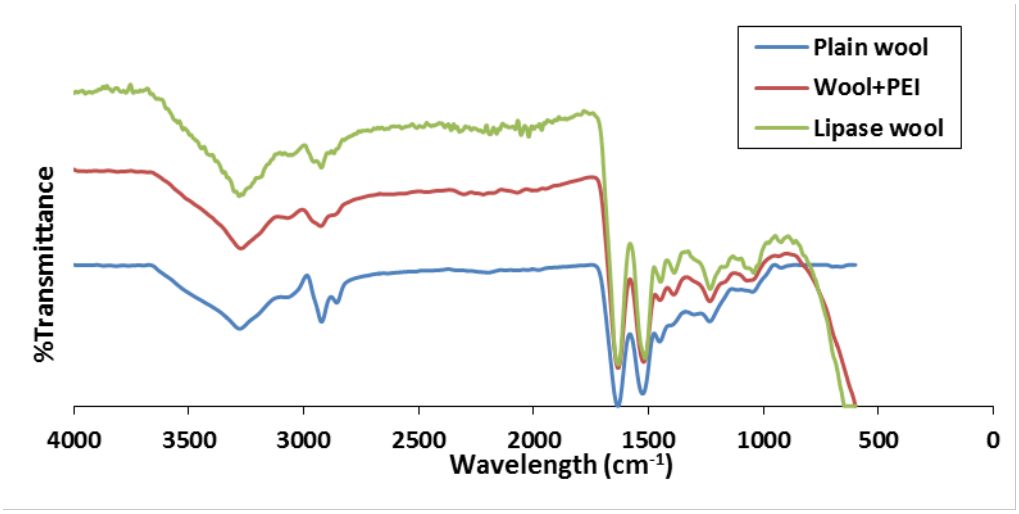


Figure B.1: FTIR spectrum of plain wool and lipase wool

SEM images of wool during different stages of lipase immobilisation

The SEM images show the morphologies of (a-b) Wool+PEI, (c-d) Wool+gluteraldehyde and (e-f)Wool+PEI+Lipase

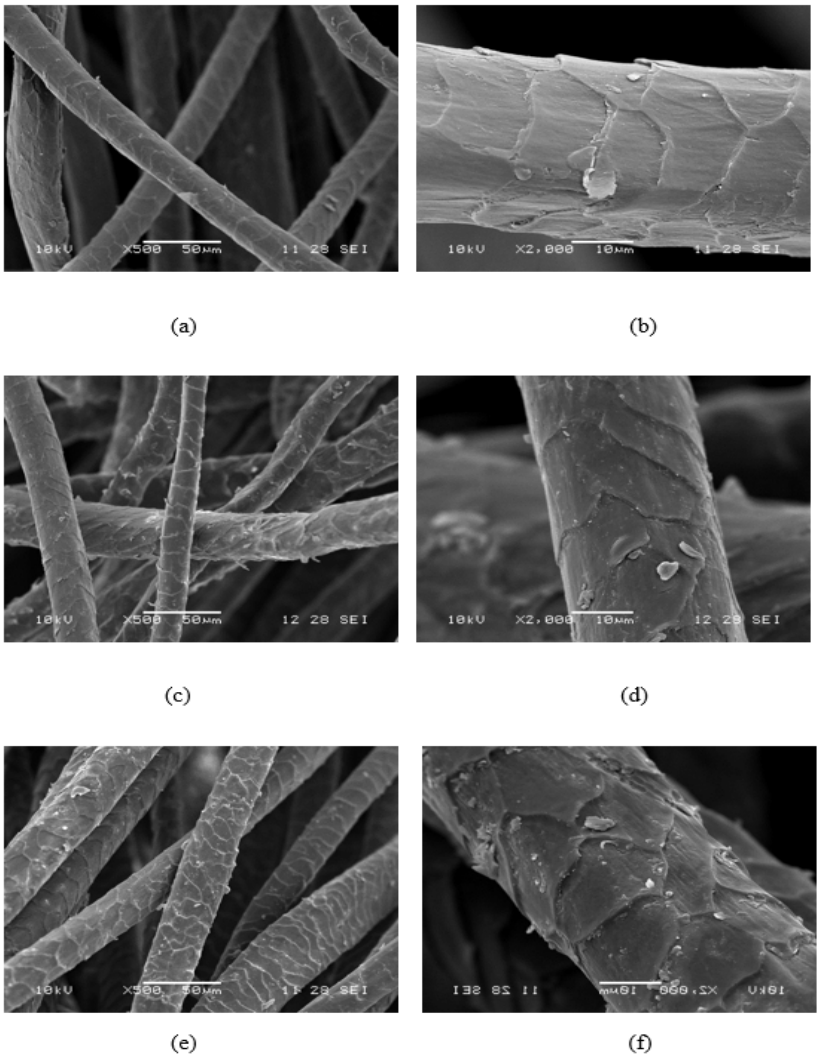
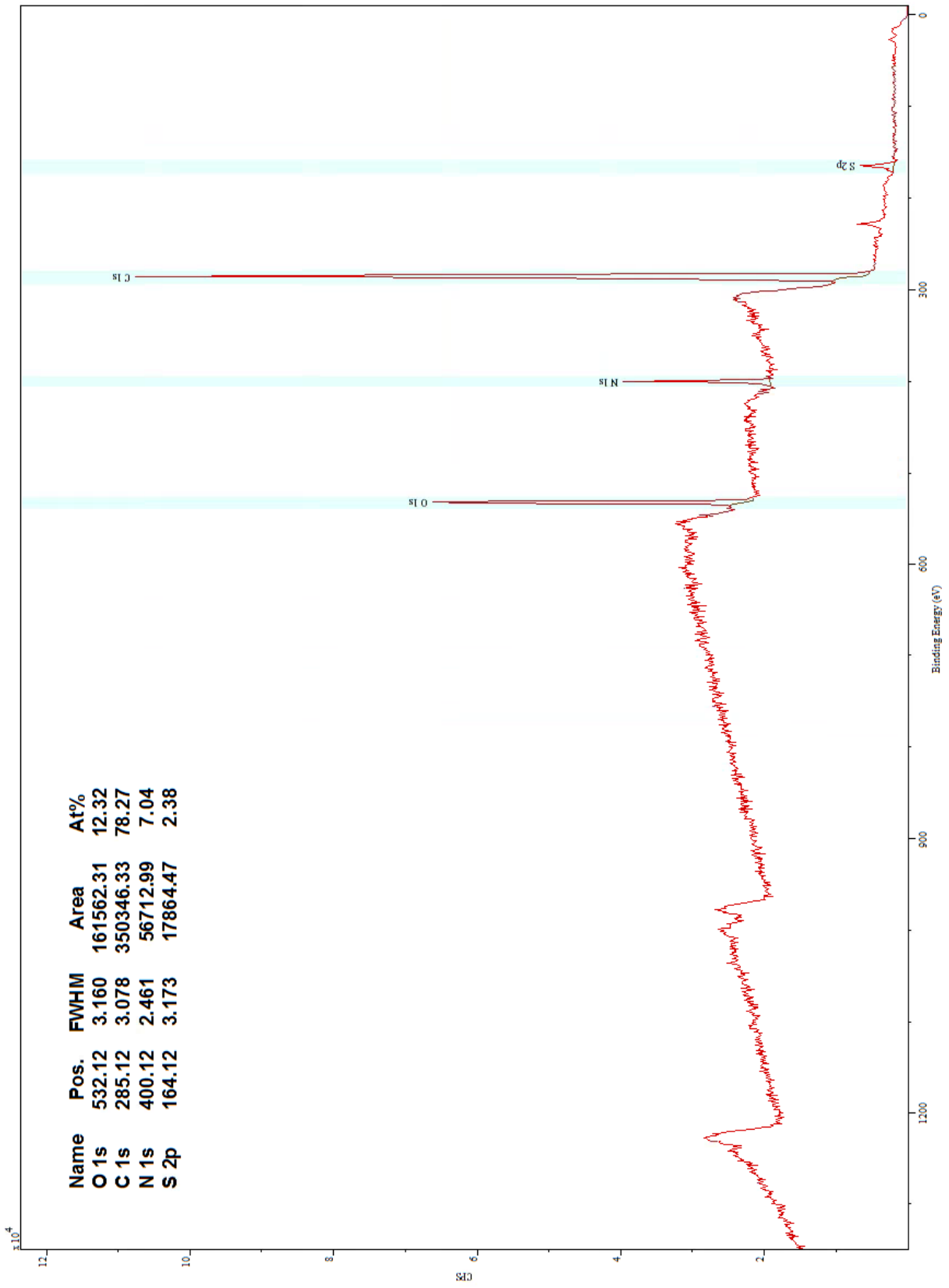
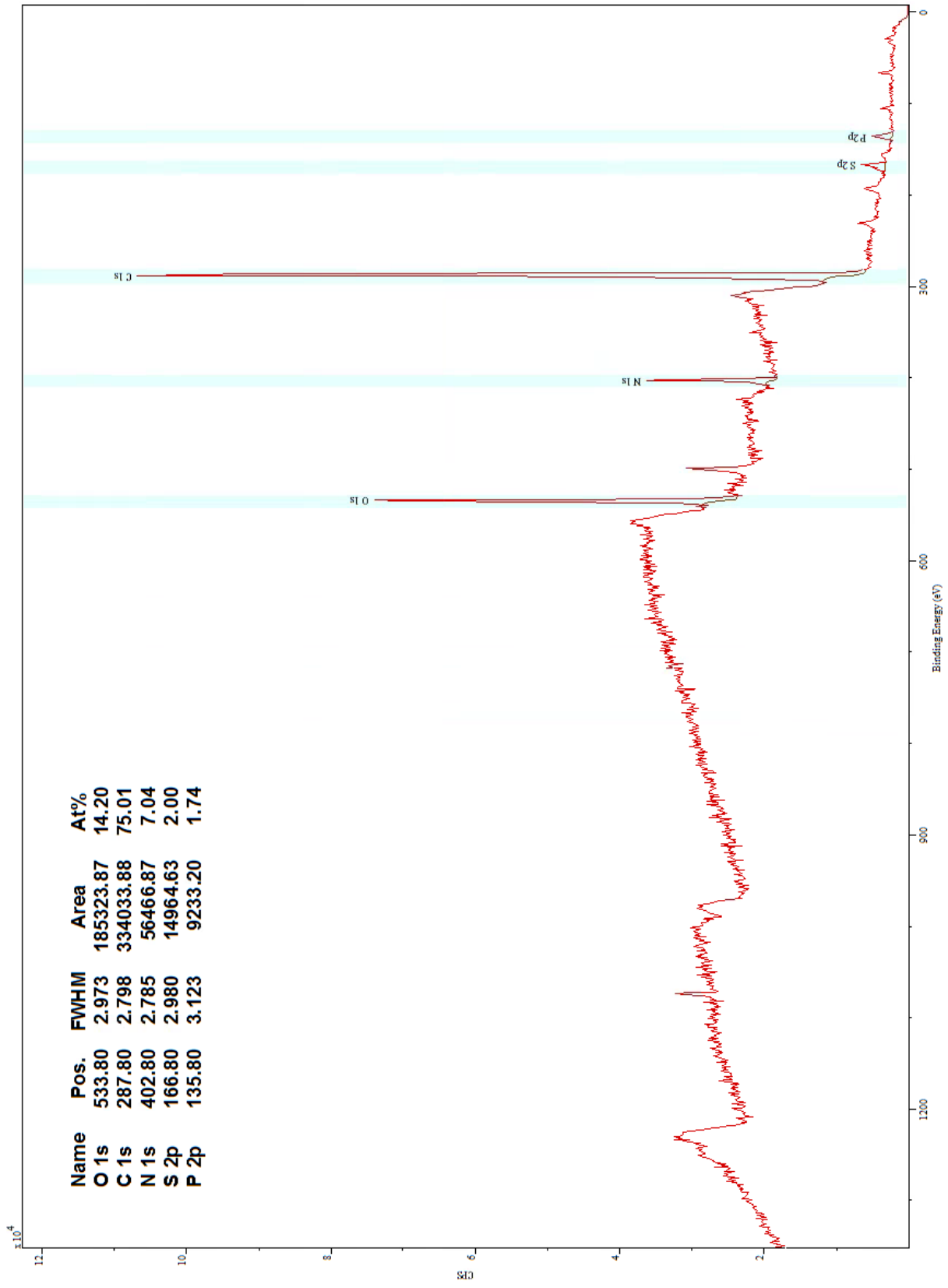


Figure B.2: SEM images at different treatment stages



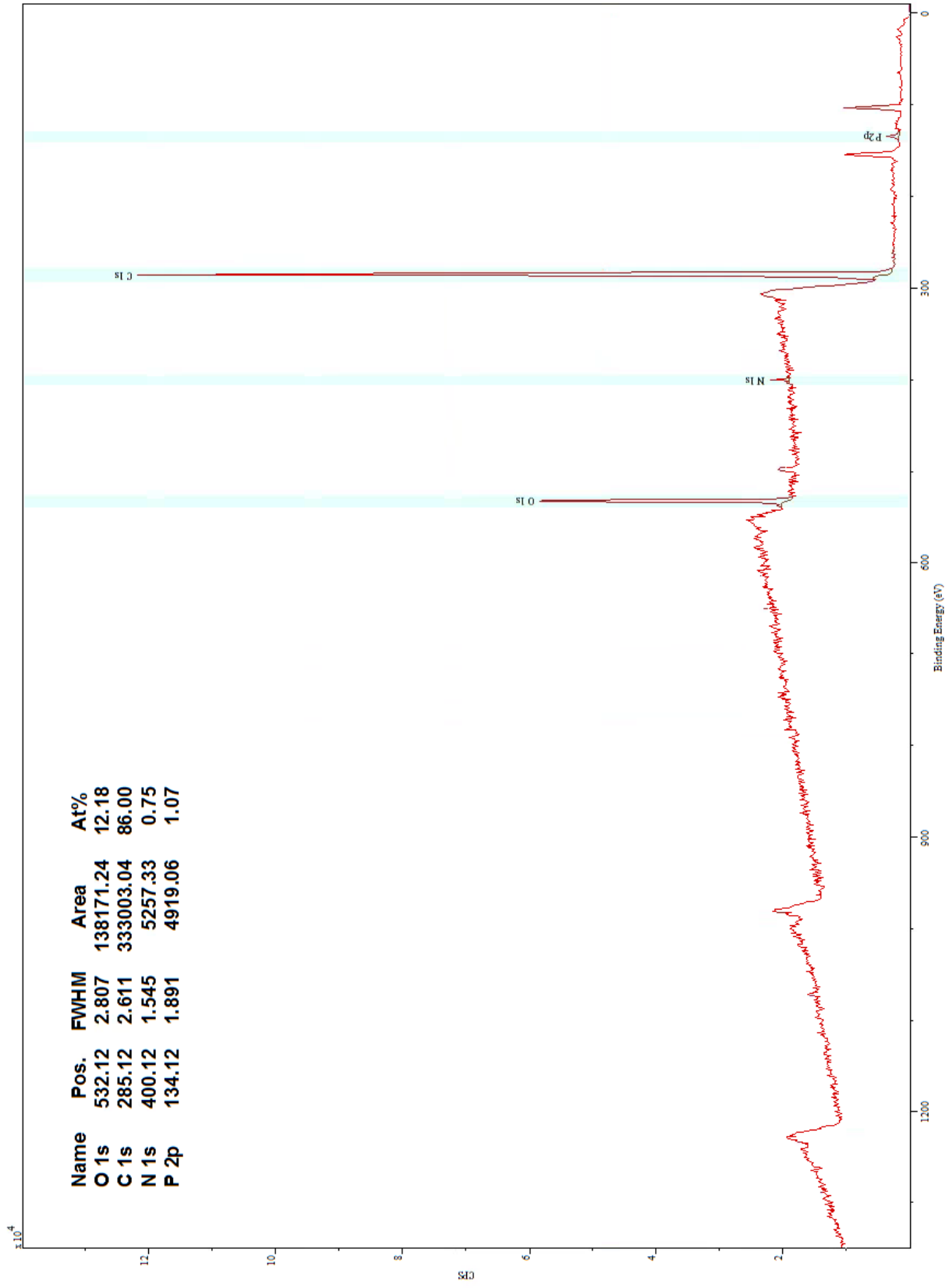
(a)

Figure B.3: Survey spectrum of plain wool from XPS



(a)

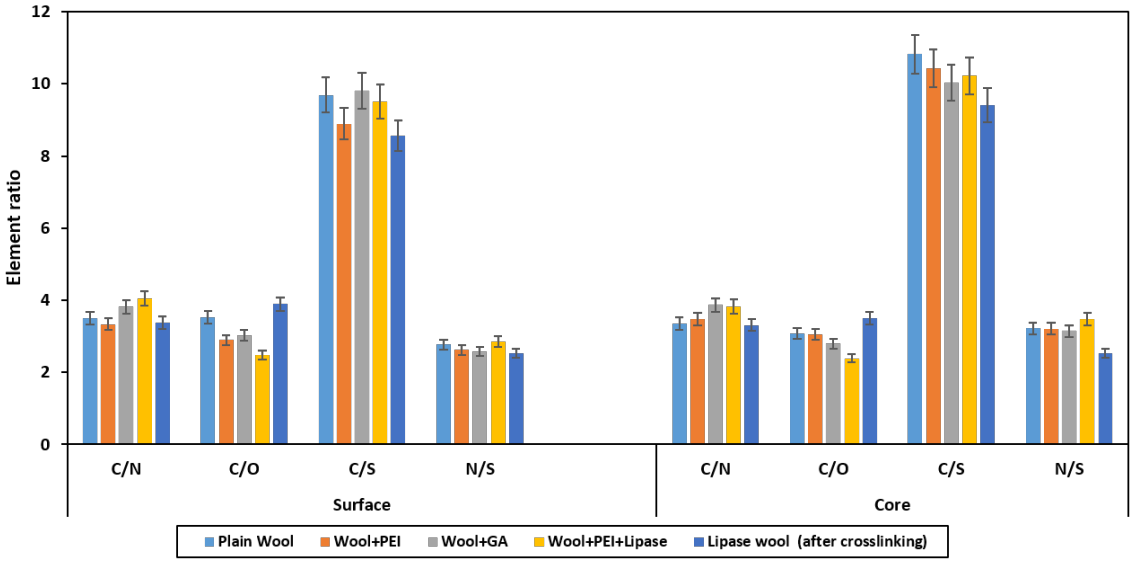
Figure B.4: (Survey spectrum of lipase wool from XPS



(a)

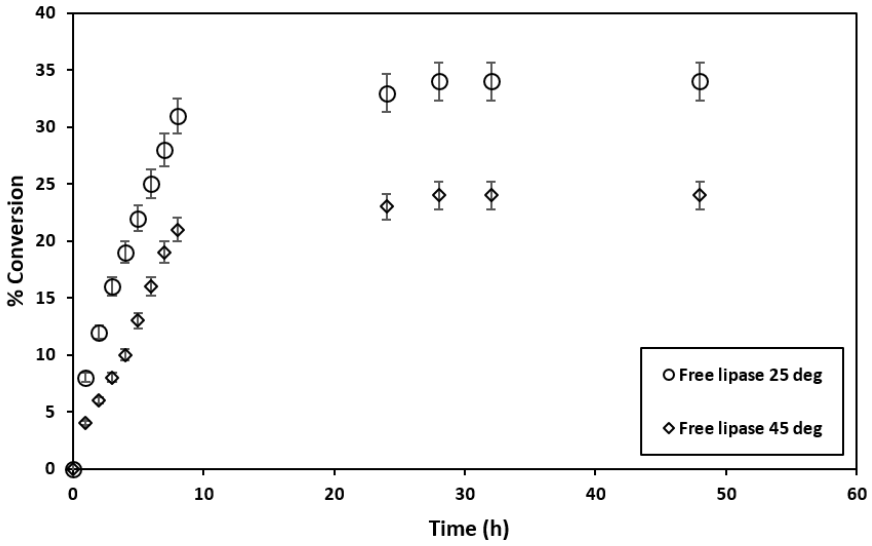
Figure B.5: Survey spectrum of lipase wool after reaction from XPS

Improved plots for Chapter 4

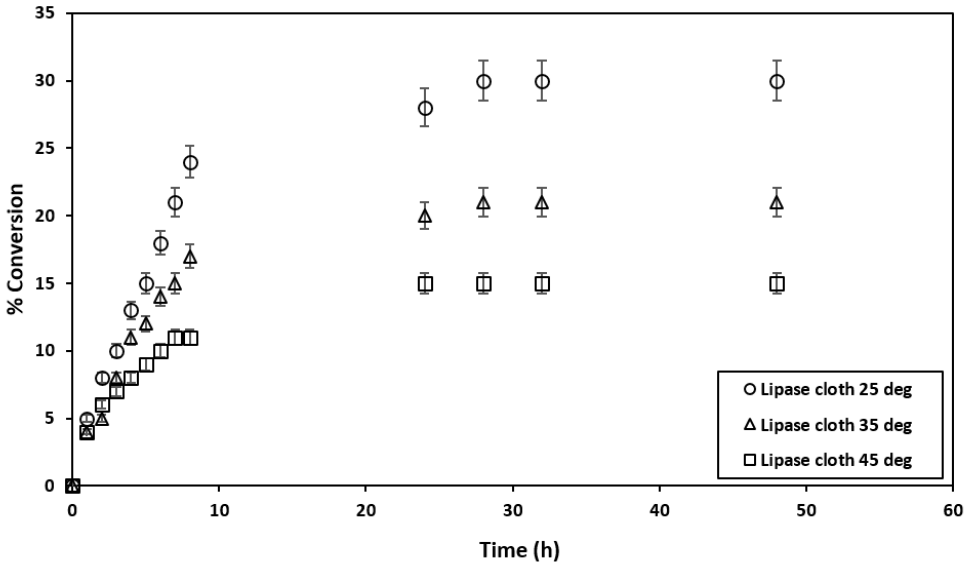


(a)

Figure B.6: Elemental ratios from the EDX analysis during different stages of lipase immobilisation

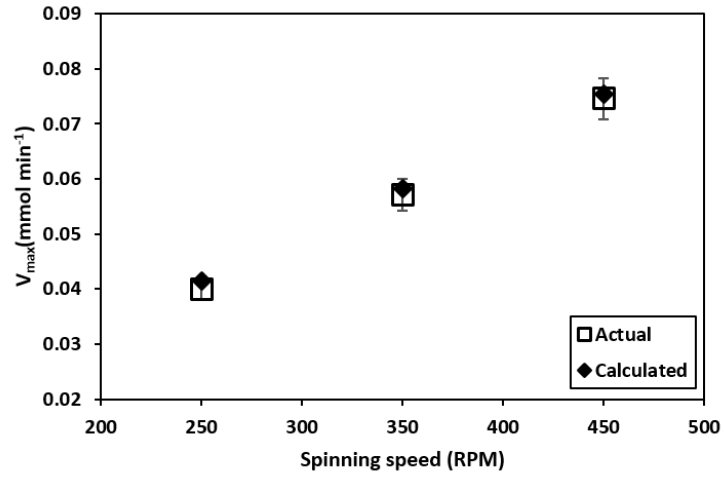


(a)

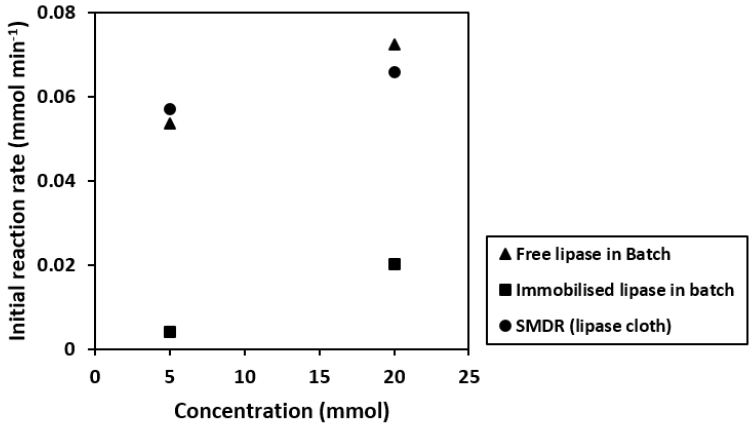


(b)

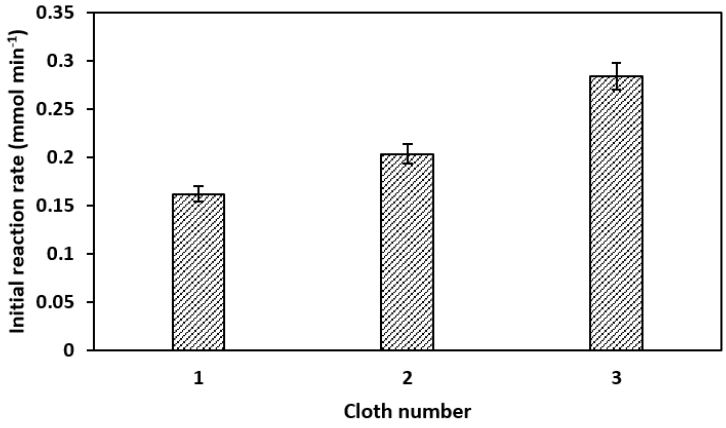
Figure B.7: Effect of temperature on reaction conversion in batch using (a) free lipase and (b) immobilised lipase



(a)



(b)



(c)

Figure B.8: (a) Experimental and calculated values for V_{max}, (b) Initial reaction rates in SMDR and batch reactor and (c) Effect of cloth number on the initial reaction rate at spinning speed of 350 RPM and flowrate of 3 ml s^{-1}

Appendix C

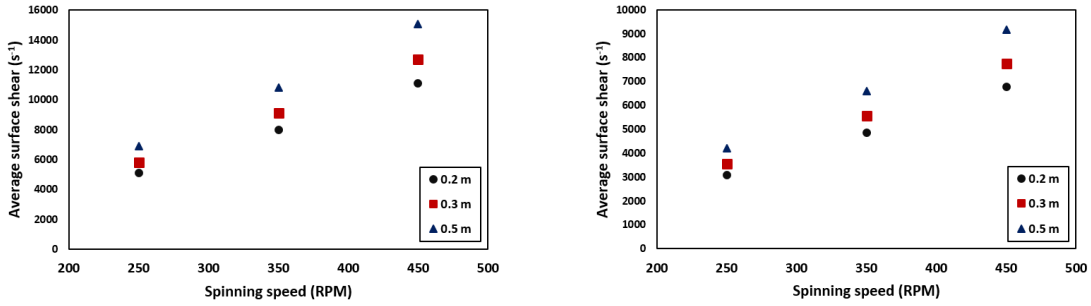
Supplementary information for Chapter 5

Surface area and volume for increasing cloth size and cloth number

Cloth size (m)	Cloth surface area (m^2)	Cloth volume (m^3)		
		1 cloth	2 cloths	3 cloths
0.2	0.1256	0.0333	0.066	0.10
0.3	0.2826	0.113	0.226	0.339
0.5	0.7856	0.523	1.046	1.57

Table C.1: Surface area and volume of cloth stack for different cloth diameters

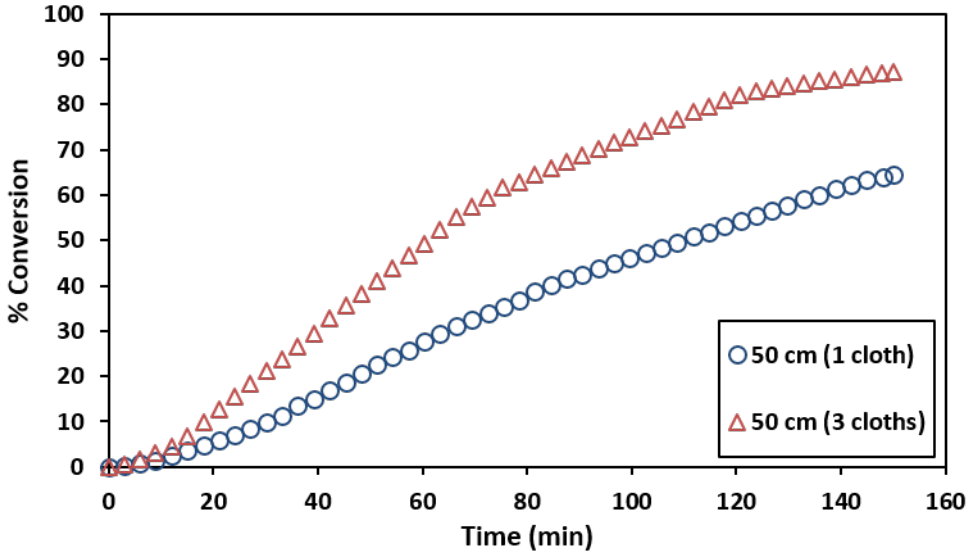
Effect of spinning speed and cloth size on average surface shear for increasing cloth diameters



(a) (b)

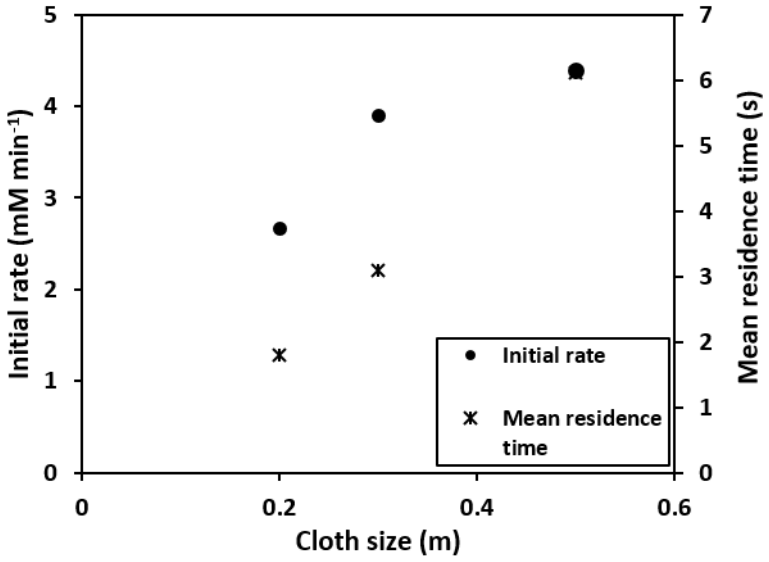
Figure C.1: (a) Average surface shear for tributyrin hydrolysis and (b) Average surface shear for nitroaldol condensation

Additional plots for Chapter 5



(a)

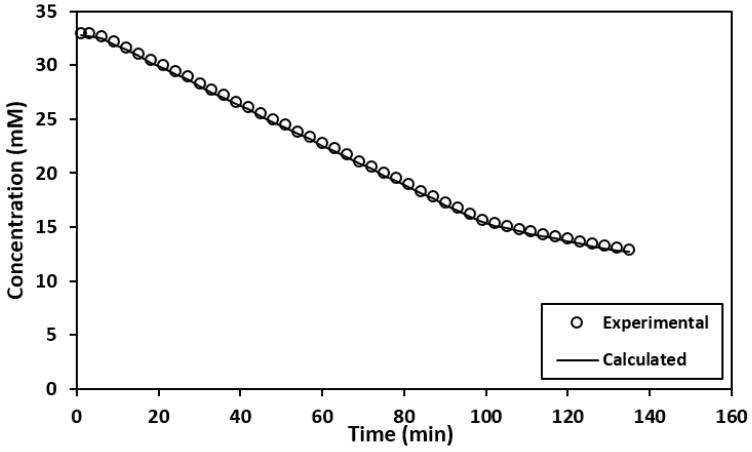
Figure C.2: Effect of cloth number on reaction rate with a substrate concemtration of 99mM. The reaction was carried out at a spinning speed of 450 RPM and flowrate of 3ml s^{-1}



(a)

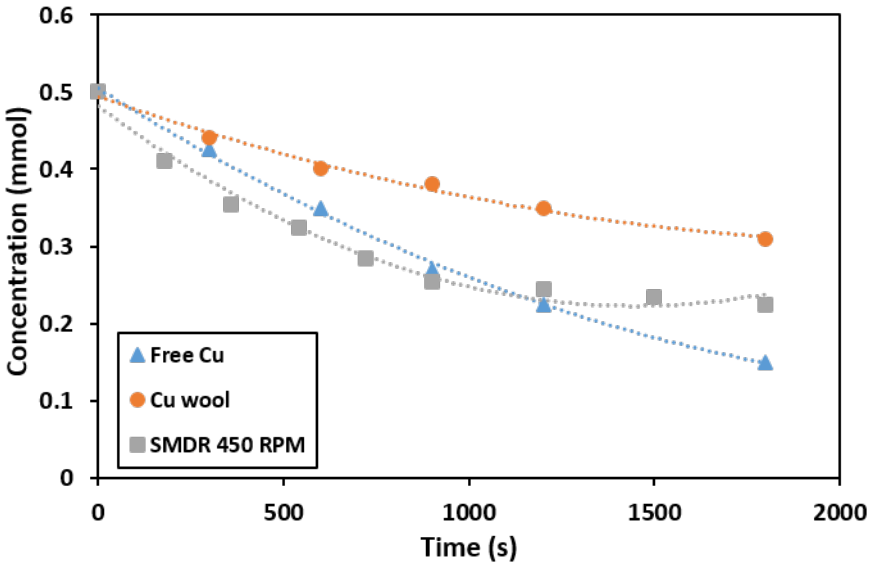
Figure C.3: Effect of residence time on reaction rate with increasing cloth size. The reaction was carried out at spinning speed of 450 RPM and flowrate of 3ml s^{-1}

The following plot shows an example for the experimental and calculated values for the time course of tributyrin hydrolysis. The theoretical change in concentration with time was calculated from the model equation proposed in Chapter-1 and k_{La} value of 0.771 s^{-1} for a flow rate of 2 ml s^{-1} and spinning speed of 350 RPM (obtained from literature). A good fit was obtained between the calculated and the experimental value, indicating the SMDR behaves like a well mixed reactor.



(a)

Figure C.4: Concentration vs time plot for homocoupling reaction in batch and SMDR



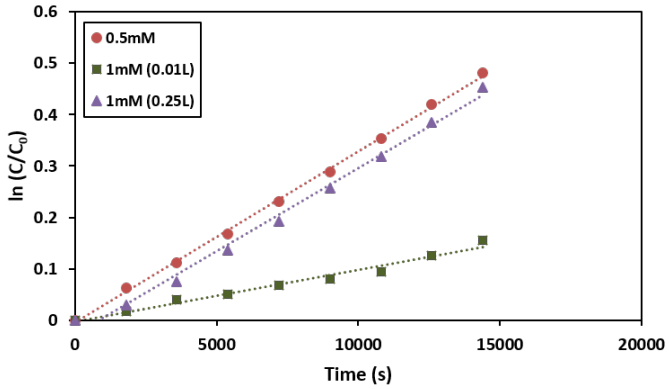
(a)

Figure C.5: Concentration vs time plot for homocoupling reaction in batch and SMDR. The initial reaction rate for the homocoupling reaction was calculated at time t=0,

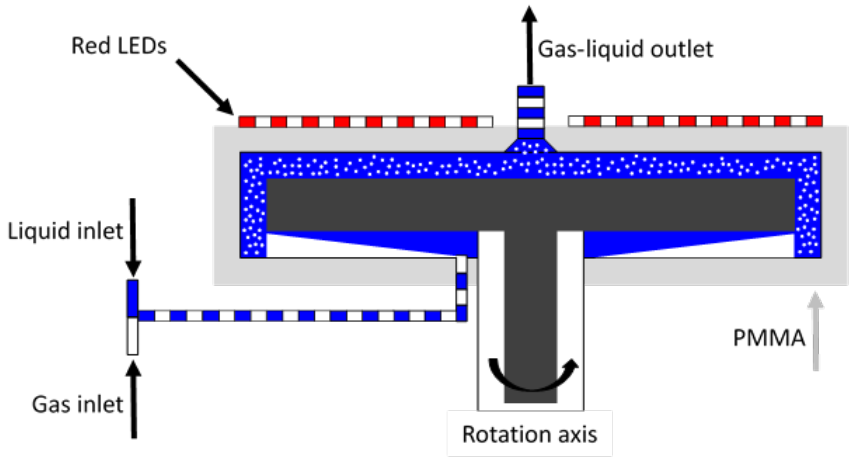
Appendix D

Supplementary information for Chapter 6

Initial tests with LED light source



(a)



(b)

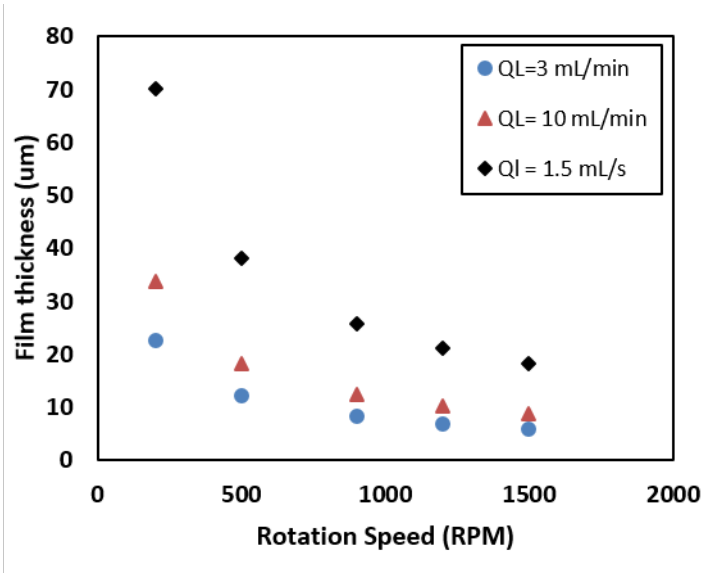
Figure D.1: (a) Reaction rate in batch at different catalyst concentrations with LED light source and (b) rs-SDR setup with red LED strips

Film thickness calculated according to Nusselt model

The film thickness was calculated as a function of spinning speed and is shown in Fig D.2

$$f = \frac{(3\mu Q)}{(2\pi\rho R^2\omega^2)}^{1/3} \tag{D.1}$$

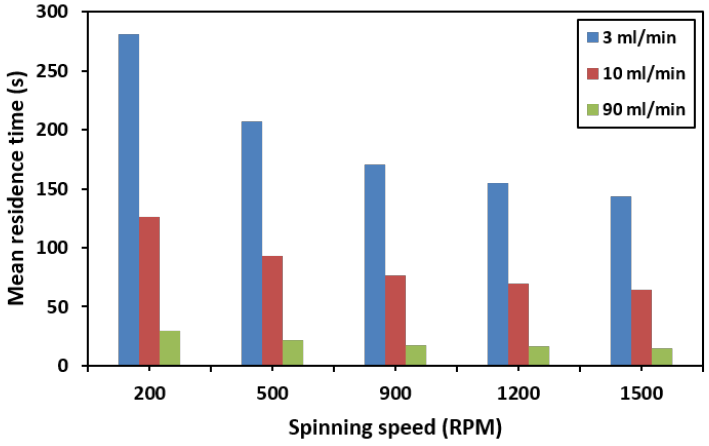
where, f = film thickness (m); μ = liquid viscosity (Pa s); Q = volumetric flow rate (m3 s^{-1}); ρ = liquid density (kg m^{-3}); R = radius of the disc (m); ω = angular velocity (rad s^{-1});



(a)

Figure D.2: Film thickness of liquid feed in rs-SDR

The mean residence time was calculated according to equation 5.6 for different spinning speeds and flowrate

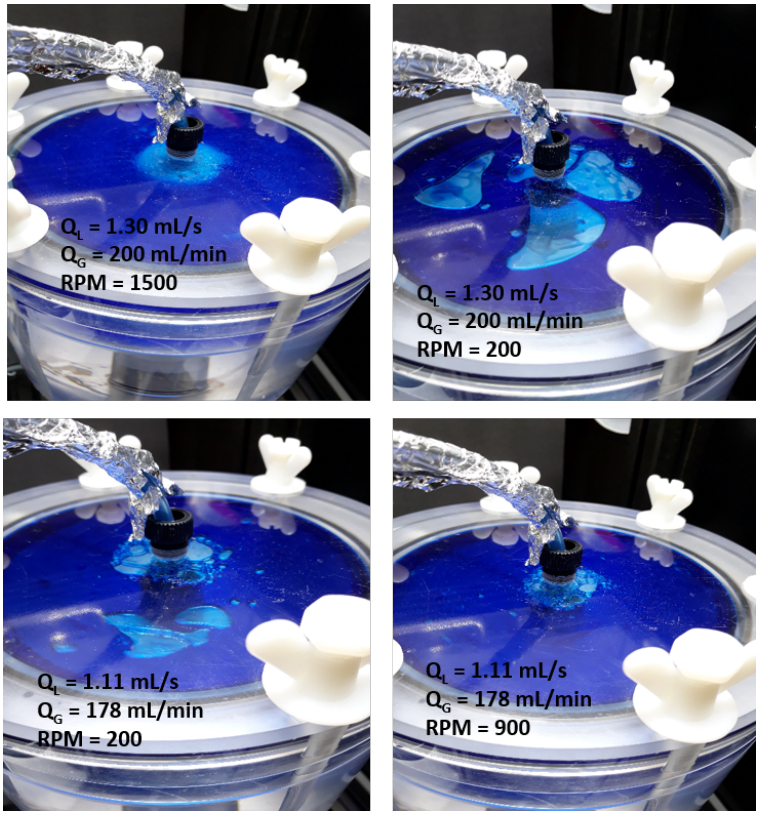


(a)

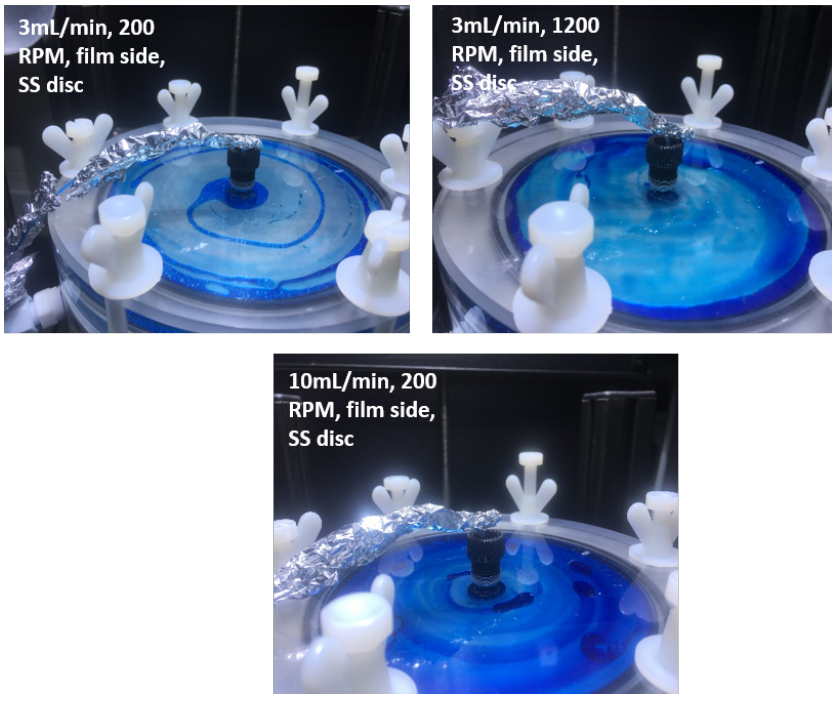
Figure D.3: Mean residence time of liquid feed in rs-SDR

Visual study in the rs-SDR - Effect of feed flowrate and rotation speed

The following figures show the different flow patterns in the rs-SDR



(a)

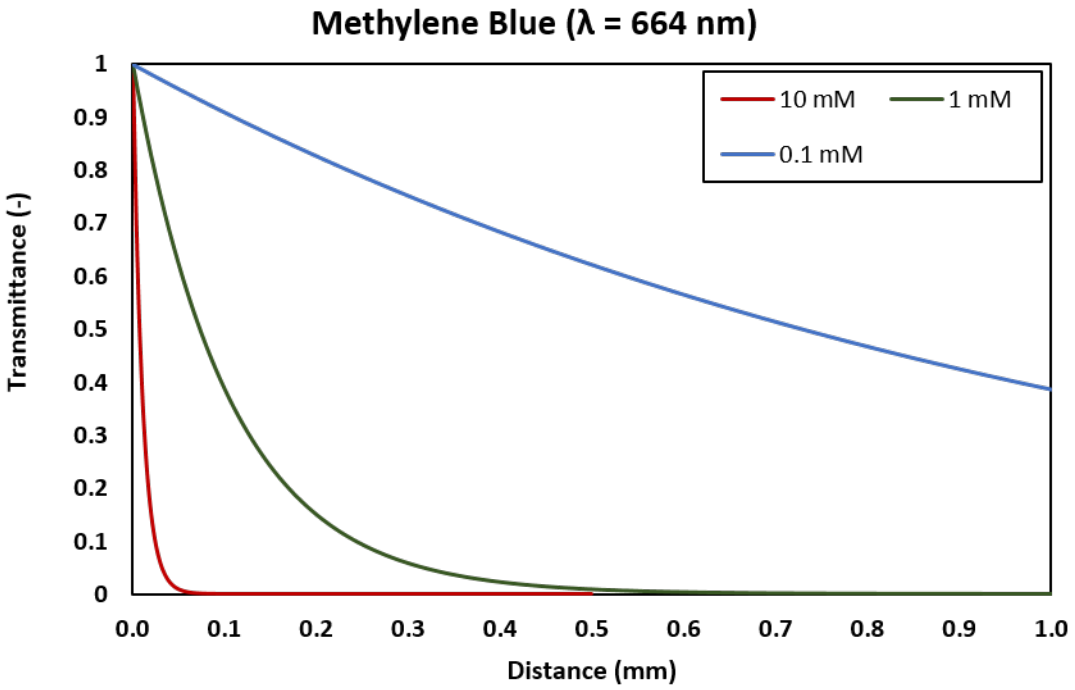


(b)

Figure D.4: Flow patterns for: (a) higher throughput and (b) lower throughput

Light transmittance for Methylene Blue

The following graph shows the effect on light transmittance with increasing liquid depth and catalyst concentration.



(a)

Figure D.5: Estimated light transmittance for methylene blue